Insilico Design and evaluation of Hybrid Hsp 90 inhibitors for Cancer Therapy

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Technology
in
Biotechnology

By
Aparna Tewari
(**212BM2361**)
Under the Guidance of
Dr. Subhankar Paul

Department of Biotechnology & Medical Engineering
National Institute of Technology
Rourkela-769008, Odisha, India
2014

Dr. Subhankar Paul
Associate Professor
Department of Biotechnology & Medical Engineering
National Institute of Technology, Rourkela, Odisha, India

Certificate

This is to certify that the thesis entitled "**Insilico Design and evaluation of Hybrid Hsp 90 inhibitors for cancer therapy**" by Aparna Tewari (212BM2361) submitted to the National Institute of Technology, Rourkela for the Degree of Master of Technology is a record of bonafide research work, carried out by her in the Department of Biotechnology and Medical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

Dr. **Subhankar Paul**
Associate Professor
Department of Biotechnology and Medical Engineering
NIT Rourkela,2014

ACKNOWLEDGEMENT

With deep regards and profound respect, I avail the opportunity to express my deep sense of gratitude and indebtedness to respected **Dr. Subhankar Paul,** Associate Professor in Department of Biotechnology & Medical Engineering, National Institute of Technology for introducing the present project topic and for his inspiring guidance, constructive criticism and valuable suggestion throughout the project work. I most gratefully acknowledge his constant encouragement and help in different ways to complete this project successfully. This project is the result of constitutive support and guidance whereby I have been accompanied many people. It is a pleasant aspect that I now have the opportunity to acknowledge all who have contributed to the work described in this thesis. This is an impossible task given the many people that have helped to design, implement, apply, and criticize the work.

Also, I take this opportunity to express sincere thanks for providing be free licensed 1 month trial pack software of Schrodinger for drug designing and virtual screening. So I am really greatful to Mr.Vinod Devaraji and Raghu Rangaswamy for providing me with valuable tool which made me accomplish my project.My veneration would be left incomplete without showing my gratification my parents, brother and almighty god for giving me an opportunity to make me contribution towards field of science and technology under their blissful showers .

I wish to express my utmost gratitude to my labmates as well as supervisors Mr. Sailendra Mahanta, Mr. Deependra Ban, Kanti K. Yadav and Sharmishtha Banerjee, Shumaila Khalid and Vikas for their deep concern, valuable suggestions, critical comments and kind co-operation

I remember with a sense of fulfilment and wish to share my happiness with all my colleagues and friends. I want to thank all my friends who have provided me with their valuable help and co-operation during my project work.

PLACE: Rourkela **APARNA TEWARI**

CONTENTS

ABSTRACT

Cancer has become most common threat to the people all over the world in which breast cancer is the second most popular cancer after lung cancer. Although innumerable research has been done for finding a suitable therapy to this disease, no cure so far has been developed. One of the main causes of breast cancer is the stabilization of number of cell signalling proteins generally known as 'client proteins' of molecular chaperones Heat shock protein (Hsp90).

Hence, inhibiting Hsp90 has been considered to be an excellent therapeutic approach in breast as well as other cancers where the diseased or stabilized proteins directed towards degradation pathway and degraded by the cellular machine proteasomal degradation system. Number of Hsp90-inhibitors has been reported so far and they are categorized into two types depending on their binding site of Hsp90. Geldanamycin which is known as N-terminal inhibitor and Novobiocin has been shown to bind C-terminal domain of Hsp90. However apart from their strong binding to Hsp90, they leave other kinds of disadvantages like solubility, and toxicity problems. In order to overcome the demerits, various analogues have been developed with a better affinity and physicochemical properties.

In our present investigation, we have developed number of hybrid inhibitor molecules using existing Hsp90-inhibitors to achieve a higher binding potential as well as minimal toxic properties. These hybrid drugs are formed by ligating two functional groups of different drugs using linker bonds which could be either amide, ester, ether, alkyl or hydrogen bonds.

To impart better molecular characteristics like intermolecular force of attraction etc. various functional groups like alcohol, amine, amide, carboxylic acid, oxyacid group were integrated. Thus wide range of hybrid drugs were designed in-silico and to evaluate their inhibitory potential to Hsp 90, molecular docking study was performed with both N-Terminal and C-Terminal domain of Hsp 90. Although docking was done at multiple sites however Lys 112 has been found to be the potential active site for hybrid drugs like H1, H2, H3, H4 and H5 in the N terminal domain of Hsp 90. Among them H1 was found to have the highest docking score of 10.116.The 2D ligand interaction analysis confirmed the involvement of four residues Glutamine 43, Asn 51, Leu 96, Ala 55 and Met 98 at the binding interface.

Later we evaluated and analysed the pharmacodynamic profile of existing drugs as well as hybrid drugs and physiochemical parameters like molecular weight, solubility, blood brain barrier permeability, cell permeability and human absorption were also reported. It was observed that hybrid drugs found to have impressive pharmaceutical profile and acceptable pharmacological potency than existing drugs. Moreover their druggability was tested on the basis of Lipinski rule of five and rule of three.

In summary, we concluded that our designed Hybrid (H1) was found to be the best Hsp 90 inhibitor among all the existing as well as designed drugs in-silico in this study. We also further state that H1 drug should be experimentally synthesized in the laboratory to evaluate its druggabillity and anti-proliferative potential in cancer to validate other results.

List of Figures

List of Table

ABBREVIATIONS

Abbreviated form	Full Form
HSP	Heat shock protein
Asn	Asparagine
Thr	Threonine
Phe	Phenylalanine
Lys	Lysine
Met	Methionine
Glu	Glutamine
His	Histidine
ADMET	Adsorption,Digestion,Metabolism,Excretion and Toxicity
H	Hybrid

CHAPTER 1

Introduction

1. Introduction

1.1 Breast Cancer

Cancer is the paradigm of series of events ranging from genetic to epigentics ranging from unrestrained replication of carcinogenic cells , angiogenesis, non stimulative antigrowth signals and pro-apoptic stimuli and escape from immune surveillance. The disease of cancer is quite epidemic type if not checked could take wild futile form .As according to WHO survey it has already tolled about 7.4 death since 2004 and still is in escalation meter worldwide .It is estimated to reach pinnacle of 12 million deaths by 2030.So there creates the emergence to have certain measure which can curtail devastating slope of cancer. (Jane et al., 2010).

In the post genomic era, diseases that are frequently caused by multiple gene abnormalities like cancer, Alzheimer disease which cannot be treated by just recognition of single novel molecular targets for cancer therapeutics as they would just provide the reduced systemic toxicity, but simultaneously have to confront with various problems being unable to recover with due to their genetic plasticity, as cancer cells are having great flexibility at adapting to a noxious environment.

Recent research reveals that in normal human body about 300-500 normal genes are mutated to oncogenic trait. Most of the time cancer are characterized by dysregulation of cell signaling pathways at multiple steps but treatment involves only single target.(Anand et al., 2008) This monotarget treatment involves high cost as well as lack of effectiveness and unassurity of cancer therapy . But anyhow if we retrench the conventional theory of one gene one drug and one disease theory and embrace on multiple drugs targeting the various sites of carcinogenicity then we can overcome the resistance in drug discovery (Park et al., 2007).

1.1.1 Traditional Treatments

Treatment of cancer involves many approaches, in which the preliminary approach was surgical treatment involving the removal of tumour but this approach is only preferred for benign and early stage cancers not for malignant cancer. Next treatment involves the use of radiation which kill cancer cells with high energy rays focusing on specific oncogenic target, but it mode of action can also involve DNA damage and thus preventing its replication of cancer cells from further proliferation. Another technique involves use of chemotherapy drugs which are toxic to the rapidly growing cells. Many of these drugs are designed to interfere with the synthesis of precursor molecules needed for DNA replication; they interfere with the ability of the cell to complete the S phase of the cell cycle. Other drugs cause extensive DNA damage, which stops replication. A class of drugs called spindle inhibitors stops cell replication early in mitosis.

1.1.2 Newer Treatments

Although cancer cells have lost some of the normal responses to growth factors, some cancer cells still require hormones for growth. Hormone therapy for cancer attempts to starve the cancer cells of these hormones. This is usually done with drugs that block the activity of the hormone, although some drugs can block synthesis of the hormone. For example, some breast cancer cells require estrogens for growth. Drugs that block the binding site for estrogens can slow the growth of these cancers. These drugs are called selective estrogen receptor modulators (SERMs) or anti-estrogens. Tamoxifen and Raloxifene are examples of this type of drug. Similarly, testosterone (an androgen hormone) stimulates some prostate cancer cells. Selective androgen receptor modulators (SARMs) are drugs that block the binding of testosterone to these cancer cells, inhibiting their growth and possibly preventing prostate cancer.

Another promising target for cancer therapy is angiogenesis. Several drugs, including some naturally occurring compounds, have the ability to inhibit angiogenesis. Two compounds in this class are angiostatin and endostatin; both are derived from naturally occurring proteins.

A technique called chemo immunotherapy attaches chemotherapy drugs to antibodies that are specific for cancer cells. The antibody then delivers the drug directly to cancer cells without harming normal cells, reducing the toxic side effects of chemotherapy. A similar strategy, radio immunotherapy, couples specific antibodies to radioactive atoms, thereby targeting the deadly radiation specifically to cancer cells.

1.2 HSP 90 and its inhibitors

One of the most abundant conserved and ubiquitously expressed molecular chaperones in eukaryotes (Bahls et al., 2002) this protein is in general responsible for stabilizing, maturation and maintenance of the conformational integrity of its 200 client proteins through its ATPase activity. It functions in coordinated manner with collaboration of co chaperones like Hsp70 and Hsp40 with its client proteins like Her2, Raf-1, Akt, Cdk4, polo-1 kinase, B-Raf,HIF-1a, , Bcr-Abl ,mutant p53 which are responsible for cell regulation and signalling.(Morgan et al., 2014).

Hsp90 inhibitors have more affinity as well as over expression for cancer cells then other somatic cells that is why it serves as more promiscuous target for anti-cancer drug development.(Jia et al., 2004).

Structurally it is the constitutive protein of 732 amino acids belonging from super family of DNA gyrase, Histidine kinase and DNA mismatch pair. It has two isomers α and β, mainly present in cytosol is homodimer whose monomer consist of four structural domains, 55 kDa the C-terminal (CTD) containing MEEVD motif and 25kDa N-terminal (NTD) containing specific ATP binding site and a middle domain (MD) connected to the NTD through an unstructured linker region.(Sreedher et al., 2013). Hsp90 exists in four different isoforms present in eukaryotic cells and plays a central role in the complex network of cellular functions. Both domains are targeted to bind to substrate polypeptides, which led to dimerization because of change in Hsp90 conformation. At dimerization state, hsp90 alpha binds to ATP in its open state and thus facilitates the attachment of co-chaperones and client protein binding. Bur when ATP hydrolyzes Hsp90 reaches closed conformation. Thus by inhibiting ATPase cycle chaperoning function of Hsp 90 is altered and mutated client protein is subsequently addressed to proteasomal degradation pathway.(Pratt et al., 2010).

Most of the Hsp90 inhibitors available are from natural sources or synthetic derivatives of natural compounds. However, there is yet to be an approved Hsp90 inhibitor drug on the market. Four different mechanisms for inhibiting the function of Hsp90 are known: (1) competitive inhibition of ATP binding, (2) targeting and disruption of Hsp90-co-chaperone interactions, (3) targeting and disruption of Hsp90-client protein interactions, and (4) interference with post-translational modification of Hsp90 (Li et al., 2009).

1.3 Computational Techniques

In todays scenario computational biology and cheminformatics are devoted to large-scale generation and analysis of information derived from 3D structures and dynamics of proteins, with the goal of scientific and commercial breakthrough in drug discovery (Kelley et.al., 2009). Rational drug design facilitates and speeds up the drug designing processes that involves various method of identifying novel compounds. One advanced method is the docking of the drug molecule or ligand or inhibitor with the target. Docking is a term used for computational schemes that attempt to find the "best" matching between two molecules: a receptor and a ligand. Docking is used to identify and optimize drug candidates by examining and modelling molecular interactions between ligands and target macromolecules. Pharmacophore library screening is followed by docking represent complimentary screening methods with the combination providing optimum results. Commonly, this screening approach is preceded by a prior filtering of virtual databases (e.g. physicochemical, ADMET/PK, stability, reactivity, toxicity, drug-like properties, etc.) (Kapetanovic et al., 2008).

1.4 Objective

- To design chimeric compounds by conjugation of two existing natural inhibitors.
- Active site prediction of full Hsp 90 protein.
- To investigate the interaction of hybrid drugs at both termini of Hsp 90 and finding the best fit poses.
- Free binding energy estimation on the basis of MMGBSA field.
- To analyze pharmacodynamic profile of each hybrid conjugates on the basis of various physiochemical parameters.

CHAPTER 2

Review of Literature

2.1 Molecular chaperones and Hsp 90

Although amino acid sequence and the laws of thermodynamics determine a protein's native conformation, the crowded cellular environment markedly influences folding of nascent polypeptide chains. The total protein concentration in the cytosol of a typical mammalian cell is ~300 mg per ml. As a result of this macromolecular crowding, newly synthesized polypeptides and previously folded proteins with low stability expose hydrophobic surfaces, constantly risking misfolding and aggregation. Both misfolding and aggregation pose a substantial burden to the organism, and protein-folding defects can give rise to numerous human diseases. Because abnormal protein folding can lead to many problems in a cell, all organisms have dedicated protein assemblies that maintain proteostasis and mitigate the life-threatening effects of heat and other stresses on the proteome. They also cooperate with the ubiquitin–proteasome system, targeting terminally misfolded proteins for degradation, and with translocation machineries to get proteins to their proper locations. Many are up regulated in response to heat and are therefore termed heat shock proteins (Hsps). Hsp90 is one of the most conserved HSPs, present from bacteria to mammals, and is an essential component of the protective heat shock response. The role of Hsp90, however, extends well beyond stress tolerance. Hsp90 uses the energy generated by ATP binding and hydrolysis to fold proteins involved in signal transduction, protein trafficking, receptor maturation (that is, the attainment of an active conformation and intracellular nature) and innate and adaptive immunity. In doing so, Hsp90 interacts with more than 20 co-chaperones, which guide its recognition of client proteins and modulate its biochemical activities. These activities are closely coupled to environmental perturbations.

Specifically, under normal conditions there are high levels of Hsp90 and thus an abundant chaperone reservoir, which buffers proteostasis against environmental stress. Under more extreme environmental conditions, the chaperone reservoir can be rapidly exhausted.Hsp90 is a large dimeric protein found in almost every compartment of eukaryotic cells. Indeed, most eukaryotic genomes encode multiple compartment-specific Hsp90 proteins, which arose early in evolution. Although structurally similar to cytosolic Hsp90, they have distinct cellular functions, and probably play important parts in health and disease Hsp90 is one of the most abundant proteins in the cytoplasm, where it constitutes 1–2% of total protein levels. (Taipale et al., 1999) Some Hsp90 translocates to the nucleus in response to stress and other stimuliHsp90 alpha functions by recruiting many co-chaperones via, Cdc37, Hop, p23, Hsp70 and Hsp40 (Li et al., 2012). The complex formation is an energy-requiring step and involves

sequential ATPase cycles (Richter et al., 2004). Client proteins of Hsp90 alpha are several kinases viz, AKT, B-Raf mutant, MET and CDK4; Transcriptional factors HIF-1A, ERA-receptors p53 mutant regulating cell proliferation and survival and chimeric fusion proteins (Theodoraki et al., 2012).

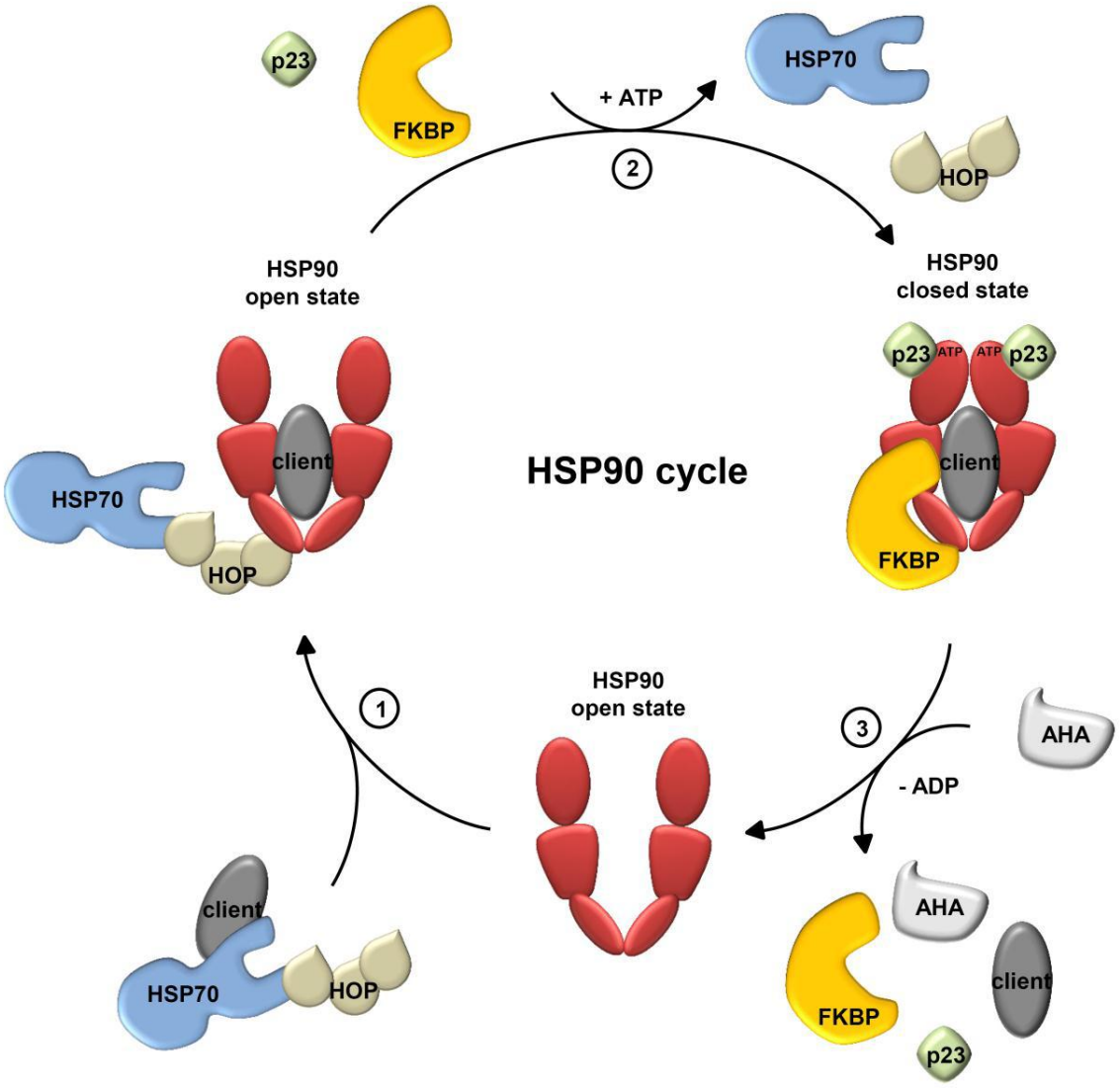


Figure 1:- **Dynamics of HSP90 and its co-chaperones (Bracher et. al.,2006).**

2.2 Hsp 90 inhibitors

HSP 90 is so named because of its molecular weight 90-kDa. It is anticipated that this molecular chaperone (Hsp90) has the most specific and most cell permeable inhibitors, and since this chaperone is the nucleation point for protein kinase-related chaperone machinery, in most cases chaperone-based kinase inhibition is achieved by using Hsp90 inhibitors.

2.2.1 Benzoquinone ansamysin class of inhibitors

Benzoquinone ansamycin is the first successful Hsp90 inhibitor which has reached the clinical trial and is most studied inhibitor, other natural products of different chemical structure have also been shown to inhibit Hsp90 both *in vitro* and *in vivo*. The first benzaquinone functional group containing drug was geldanamycin which is isolated from Streptomyces hygroscopicus (Supko et al., 2005). Though the antitumor effects of geldanamycin were initially thought to be due to specific tyrosine kinase inhibition, later studies revealed that the antitumor potential relies on depletion of oncogenic protein kinases via the proteasome (Neckers et al., 2007) Structural and biochemical studies have demonstrated that GA is a competitive inhibitor of ATP binding to Hsp90 (Roe et. al.2003). Binding of GA in the N-terminal ATP pocket restrains Hsp90 in its ADP-bound conformation and prevents the subsequent "clamping" of Hsp90 around a client protein (Blagg et al., 2003) resulting in ubiquitination and proteasomal degradation of the client (Mimnaugh et al., 2006) Although GA exhibited potent anti-cancer activities in preclinical studies, it had little clinical potential mostly due to the high hepatotoxicity observed in animal models. As a result, GA derivatives that maintain similar anti-cancer activities but with better toxicological properties were synthesized, such as 17-AAG (17-allylamino-17 desmethoxygeldanamycin; tanespimycin, KOS-953) (Workman et. al., 1995) 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin) (Workman et al., 1995) and IPI-504 (17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride) several drawbacks of 17-AAG, including low water-solubility, instability in solution, and low oral bioavailability may be the major obstacle to further clinical application (McCollum et al., 2002).

In addition to 17-AAG, other GA derivatives have been developed for clinical use as well. Currently 17-DMAG, a more water-soluble analogue of 17-AAG, has entered Phase I and Phase II clinical testing, and displayed higher oral bioavailability, lower toxicity, and

increased stability compared with 17-AAG (Ronnen et al., 2007). Another water-soluble hydroquinone hydrochloride analogue of 17-AAG is IPI-504 (Grbovic et al., 2008). IPI-504 is in Phase I and Phase II clinical trials to evaluate its potential for treating cancer that has become resistant to therapy with tyrosine kinase inhibitors.

2.2.2 Resorcinol class of inhibitors

Radicicol is a macrocyclic antibiotic isolated from Monosporium bonorden. Because of its potential to reverse the malignant phenotype like geldanamycin (Ronnen et al., 2009). It was initially thought to be a tyrosine kinase inhibitor. However, later studies showed its role in Hsp90 client protein degradation [Heath et al., 2006]. Radicicol is involved in the degradation of NQQ1, followed by degradation of mutant p53, which is involved in the malignant transformation. Thus, radicicol and its derivatives also bind in the nucleotide pocket of Hsp90, with nearly identical biological effects as 17AAG (Grbovic et al., 2008).

In past far more than 200 client proteins have been identified by using these natural products like geldanamycin and radicicol. But they have always been accompanied with some perils, though they have been evaluated in clinical trials, but these none of the 1st generation of Hsp90 inhibitors has circumvented their limitations in pharmacodynamics profiles. However, recent studies have portrayed that some new agents, 2nd generation of Hsp90 inhibitors with different chemical makeup from geldanamycin and radicicol, have entered clinical trials but still only some of them showed clinical efficacy.

2.2.3 Aminocoumarin class of inhibitors

The aminocoumarins novobiocin, chlorobiocin and coumermycin A1 are structurally related antibiotics produced by different Streptomyces strains. They are potent inhibitors of bacterial gyrase. Their binding sites and their mode of action differ from those of fluoroquinolones such as ciprofloxacin. Novobiocin has been introduced into clinical use against Staphylococcus aureus infections, and S. aureus gyrase is particularly sensitive to inhibition by aminocoumarins, while topoisomerase IV is much less sensitive. (Heide L.et al., 2004)

Structurally, novobiocin and the closely related aminocoumarin clorobiocin (composed of a 3-dimethylallyl-4- hydroxybenzoyl moiety, a 3-amino-4,7-dihydroxycoumarin moiety (ring

B) substituted with a methyl group and a chlorine atom, respectively, and a substituted deoxysugar . The 3″-OH of the deoxysugar is esterified with a carbamoyl group in the case of novobiocin and with a 5-methylpyrrole-2-carboxyl moiety in the case of clorobiocin. In contrast to the carbamoyl group of novobiocin, the 5-methylpyrrole moiety of clorobiocin is able to occupy an additional hydrophobic pocket in the GyrB subunit and to displace two water molecules. Thereby, clorobiocin binds more effectively to the GyrB subunit than novobiocin. (Anderle et al., 2007).

Likewise, novobiocin and other coumarin antibiotics bind to the carboxyl terminus of Hsp90 and also disrupt its ability to chaperone client proteins. Whether either of these classes of natural products will produce a better alternative to 17AAG remains to be seen, but the possibility remains that, if the dose-limiting toxicity. (Neckers et al., 1998)

2.2.4 Anthracycline class of inhibitors

Anthracyclines are anticancer compounds that were originally derived from Streptomyces and their anti-tumor activities were established in the 1960s. Anthracyclines are red aromatic polyketides and occur in variety of forms due to the structural differences in the aglycone and the different sugar residues attached.

Daunomycin (daunorubicin) was the first anthracycline compound to be chatacterized structurally and stereochemically(Cortes-Funes et al.,2005). Danorubicin is used in treating acute lymphoblastic and myeloblastic leukaemias Adriamycin (generic name doxorubicin) is a hydroxyl derivative of daunorubicin. Doxorubicin is one of the most widely used chemotherapeutic agents and is generally prescribed in combination with other drugs. Doxorubicin has a broad spectrum of activity. It is one of the most effective drugs for solid tumor treatment, e.g., breast cancer, small cell lung cancer and ovarian carcinoma treatments. Epirubicin is an epimer of doxorubicin and differs only in the orientation of the C-4 hydroxyl group on the sugar. Because of this slight change in the structure, epirubicin has lower cardiotoxicity than doxorubicin. Epirubicin is used in the treatment of gastric and breast cancer and is also indicated for the treatment of carcinoid, endometrial, lung, ovarian, esophageal and prostate cancers as well as soft tissue sarcomas. Idarubicin is an analog of daunorubicin. It lacks the C-4 methoxy group and this increases its lipophilicity. Idarubicin has improved activity as induction therapy for acute myelogenous leukaemia (Arcamone et al., 2005) like any other genotoxic agent, doxorubicin has been demonstrated to induce the

binding of p53 to DNA. As p53 is a major player in some forms of apoptosis, it has been proposed that anthracyclines may exert3 their cytotoxic effect via p53 mediated apoptosis (Brockmann et al., 1998). The side effects of anthracyclines, like any other chemotherapeutic agent, are linked to their cytotoxicity to non-differentiated, proliferating normal cells. However, the major toxicities of anthracyclines include cardiotoxicity and myelosuppression and these are the major limitations of these drugs. Doxorubicin can also cause severe local tissue necrosis. Cardiomyopathy and congestive heart failure are the two cardiotoxic side effects of anthracyclines. Epirubicin is less cardiotoxic than doxorubicin but may not totally eliminate the risk of chronic cardiotoxicity (Arcamon et al., 2001).

2.2.5 Flavonoids class of inhibitors

Flavonoids are part of this family & have more than 4000 varieties. They have been classified according to their molecular structure that consists of two benzene rings joined by a linear three-carbon chain and forms an oxygenated heterocycle (C6-C3-C6) and their large number arises from the various combinations of multiple hydroxyl, methoxyl, and O-glycoside group substituents on the basic benzo--pyrone (C6-C3-C6) moiety.(Chahar et al., 1999).

Derrubone is a prenylated isoflavone that was first isolated from Derris robusta in 1972 (Hastings et al., 1972) Derrubone was originally isolated and characterized as one of a series of structurally related isoflavonoids derived from theIndian tree Derris robusta. Isolation from this source provided four isoflavones (including 1) and five 3-aryl-4-hydroxycoumarins related to Derrubone's ability to inhibit Hsp90 and not Hsp70, which coassemble to form a competent heteroprotein complex with luciferase in reticulocyte lysate, was determined using purified recombinant Hsp70. Accordingly, derrubone (Hadden et al., 2003) has been identified as a natural product inhibitor of the Hsp90 protein folding machinery. It inhibits Hsp90-dependent refolding of luciferase and antagonizes the ability of the Hsp90-specific inhibitor geldanamycin to disrupt complexes formed between Hsp90, Cdc37, and their client kinase, HRI. Furthermore, it exhibits potent antiproliferation and Her2 degradation in human breast cancer cell lines as well as it specifically downregulates numerous Hsp90 client proteins in a concentration dependent manner. The identification of derrubone as an Hsp90 inhibitor provides a new natural product scaffold upon which the development of improved Hsp90 inhibitors can be pursued (Hadden et al., 1997).

2.2.5.1 EGCG

Catechins from green tea belong to the family of flavonoids that are powerful antioxidants and free iron scavengers. Many botanical flavonoids possess strong antioxidant activities in the cardiovascular system. Effects of green tea on cancer chemoprevention have been attributed to its antioxidant activities. Structure-activity relationships of tea polyphenols on cancer chemoprevention were also explored. The main active compounds in green tea are polyphenols. In this study, 10 representative tea polyphenols were selected to evaluate their biological activity. Based on their chemical structures, they were separated into two groups, phenolic acids and flavonoids, the latter of which was separated into two subgroups, the flavan-3-ol unit group and the galloylated catechin group (Du et al., 2012).

2.2.6 Purine based class of inhibitors:-

Hsp90 contains a conserved N-terminal ATP/ADP binding pocket and nucleotide binding regulates the chaperone function of the protein. Earlier studies with various Hsp90 inhibitors showed that most of them bind directly to the N terminal ATP/ADP site, resulting in a change of Hsp90 conformation and a consequent interference with its chaperone function. In a recent development, PU3, a purine- based Hsp90 inhibitor was designed using X-ray crystallographic data. PU3 behaves like geldanamycin in inhibiting Hsp90 client protein degradation, and in possessing a robust antitumor potential. Attempts to modify and improve PU3 led to the development of PU24F-Cl, which binds to the N-terminus of Hsp90 with a 30-fold higher affinity than the parent compound, PU3, thus approximating the binding affinity of 17AAG. PU24F-Cl was found more selective than Hsp90 inhibitors. Its water solubility is also an advantage over geldanamycin and 17AAG. However, PU24F-Cl may not show the specific intracellular accumulation typical for the more hydrophobic geldanamycin analogues (Sreedharet et al., 2004).

2.2.7 Triterpenoid class of inhibitors

Triterpenoids are ubiquitous in the plant kingdom. Recent evidences support the beneficial effects of naturally occurring triterpenoids against several types of human diseases, including various cancers. Here, we have summarized the potential of triterpenoids belonging to the

lupane, oleanane, ursane, and cucurbitacin groups, and their beneficial effects based on both laboratory and clinical investigations. Anticancer potential of triterpenoids and their anti-inflammatory, anti-proliferative, and pro-apoptotic effects have been discussed both in in vitro and in vivo models. Importantly, a large number of preclinical efficacy studies using chemically-induced, as well as tumor xenograft models provided evidence that both naturally occurring and synthetic derivatives had chemopreventive and therapeutic effects. In this review, we have highlighted several studies on chemopreventive and anticancer potential of triterpenoids based on various preclinical animal models of colon, breast, prostate, and melanoma cancers (Patlolla et al., 2012).

Celastrol, also called tripterine, is a quinone methide triterpenoid isolated from the Chinese plant Tripterygium wilfordii Hook F (TWHF), which has been used as an anti-rheumatic in China for many years. Celastrol can activate HSF1, induce expression of some HSPs, down-regulate HSP90's ability in binding to ATP and disrupt the combination of HSP90 with co-chaperone Cdc37. All these effects indicate inhibition of HSP90 activities. In agreement with data on the anti-tumour effects of other HSP90 inhibitors, celastrol showed similar action upon a variety of tumour cells. Moreover, using in silico screens of public gene expression data, celastrol has recently been discovered to eradicate acute myelogenous leukemia stem cells through simultaneous inhibition of NF-κ B-mediated survival signals and induction of oxidative stress. It is therefore possible that when compared to other HSP90 inhibitors celastrol possesses unique anti-tumor properties.(Peng et al., 2011).

2.3 Combinational Hybrid Theory

The concept of "hybrid drugs" has been gaining popularity in medicine. Since a single drug is not always able to adequately control the illness, the combination of drugs with different pharmacotherapeutic profile may be needed .Drugs involving the incorporation of two drug pharmacophores in a single molecule with the intention of exerting dual drug action have been described . For example, one of the hybrid parts may be incorporated to counterbalance the known side effects associated with the other hybrid part, or to amplify its effects through action on another biological target. Ultimately, no matter how familiar the building blocks

may be, hybrid drug molecules may, at their core, become new molecules with identities independent of their precursors.

Hybrid molecules with dual functionality development and/or multitherapeutic strategies, which utilize new chemical entities with two (or more) different heterocyclic skeletons (pharmacophores), represent a valid and rational approach in design and development of novel Hsp 90 inhibitors. These drugs have the potential to surmount the rapid development of resistance, enhance patient compliance, and reduce both the cost and the risk of drug–drug interactions.(Muregi et al, 2012). Blagg and coworkers reported the design synthesis and biological evaluation of some chimeric compounds bearing both the quinone moiety present in geldanamycin and resorcinol groups present in radicicol; the new hybrid compounds proved to be endowed with better binding affinity and improved inhibitory activity against cancer. More recently, Walsh and coworkers combined fast-acting artemisinin and slow-acting quinine into a hybrid drug for malaria, for which drug resistance is a barrier to effective treatment. In vitro assays showed that the hybrid is more effective against drug-sensitive and drug-resistant malaria than the individual drugs alone or a cocktail made of a 1:1 molar ratio of the two. Walsh suggested that the hybrid drug may increase cellular uptake which improves the treatment's efficacy, Vangapandu and co-workers demonstrated that 8-quinolineamines conjugates as well as their "double prodrugs" had promising in vivo activity in mice. If these compounds, in which basic pharmacophore is primaquine, are modified to improve their blood schizontocide activity, they have the potential to be used as broad-spectrum (tissue and blood schizontocides) antimalarial agents.

Conjugation of drugs may, therefore, serve as a useful tool to improve drug solubility and stability, and prolong drug release, reduce doses, dosing intervals, and drug toxicity, as well as to achieve targetability (Saadeh et al., 2013).

However, based on the wide interest in the hybrid molecules as well as numerous encouraging efficacy and toxicity reports, the next generation of Hsp 90 inhibitor may as well be hybrid drugs as opposed to multi-component ones. There are numerous advantages of employing hybrid molecules over multicomponent drugs in cancer therapy. Compared to the latter, hybrid drugs may be less expensive since, in principle, the risks and costs involved may not be different from any other single entity. Another advantage is that of the lower risk of drug–drug adverse interactions compared to multicomponent drugs. The downside,

however, is that it is more difficult to adjust the ratio of activities at the different targets (Morphy et al., 2005). Different types of hybrid molecules could be classified as :-

1. ***Conjugates*** In which the molecular frameworks, that contain the pharmacophores for each target are separated by a distinct linker group that is not found in either of the individual drugs. Most conjugates contain a metabolically stable linker (Morphy et al., 2005).

2. ***Cleavage conjugates*** have a linker 1designed to be metabolized to release the two drugs that interact independently with each target.

3. ***Fused hybrid*** molecules have the size of the linker decreased such that the framework of the pharmacophores is essentially touching.

4. ***Merged hybrids*** have their frameworks merged by taking advantage of commonalities in the structures of the starting compounds, which give rise to smaller and simpler molecules (Morphy et.al, 2005).

5. ***Drug-delivery system:*** As the search for novel7drugs continues, more drugs with novel targets are being developed, but many of these do not reach clinical trials due to associated toxicity. Hybrid molecules are essentially prodrugs that aid in improving the efficacy and reducing the toxicity and other adverse effects of drugs by controlling their pharmacokinetic properties (Vangapandu et al., 2003).

CHAPTER 3

Tools and Methods

3. Tools and methodology

3.1 Various databases, tools and software's used in research

1. RCSB-PDB (www.rcsb.org)
2. Pubmed NCBI (www.ncbi.nlm.nih.gov/pubmed)
3. Uniprot (www.uniprot.org)
4. BLAST
5. Clustal W
6. Phyre server
7. Open babel software 2.3.1
8. PRODRG 2.5 server(beta)
9. Chemsketch tool provided by ACD Labs
10. Chimera visualization tool
11. Docking tool:- Glide (Schrodinger software, Maestro 9.6)
12. ADMET prediction tool:- Qik Prop (Schrodinger software, Maestro 9.6)(Kind gift from Raghu Rangaswamy, Executive Director, Schrodinger, www.Schrodinger.Com, Innovations In Computational Chemistry, Bangluru).

3.2 Extraction of protein sequence from UNIPROT

UniProt is a comprehensive, high quality and freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. The UniProt consortium comprises the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource(PIR). Its URL address is (www.uniprot.org).

1. The above mentioned URL was browsed.

2. In search option, Hsp90 alpha was typed.

3. There were many results of the search but the human species type of Hsp 90 alpha of isoform 2 was selected and its fasta sequence was selected.

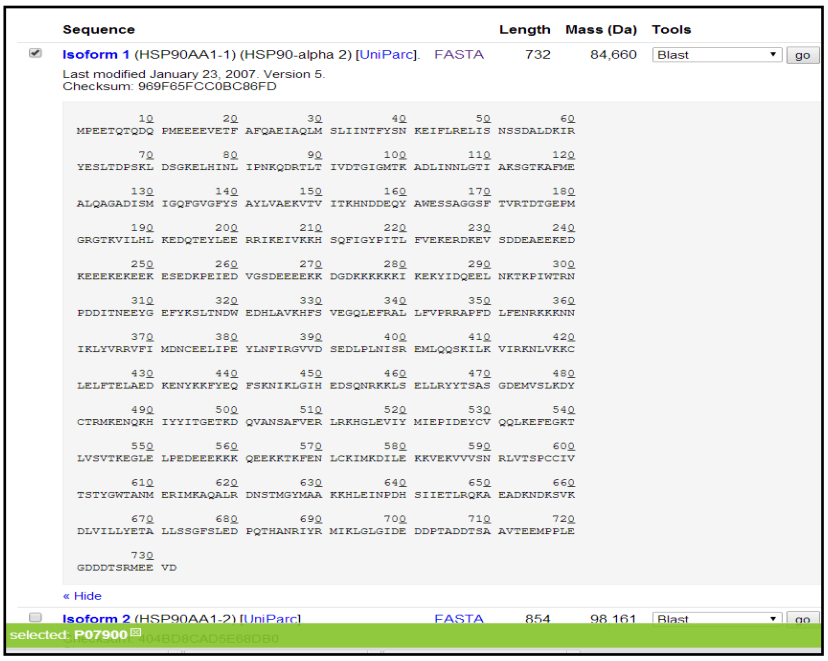


Figure 2 : Fasta protein sequence selected from uniprot for Hsp 90 modelling.

3.3 Hsp 90 protein modelling from Phyre server

PHYRE is an automatic fold recognition server used for calculating the structure and function of the protein sequence which has been entered in the serverx. It works on the principle of Homology Modeling and based on Hidden Markov Models. It is basics primarily used only for academic purpose.

1. Phyre server webpage was browsed with link address (www.sbg.bio.ic.ac.uk/phyre2)
2. E-mail id and project title is submitted.
3. Fasta sequence of the protein if pasted and the job is allowed to run for approximately 5 hrs and results are send to email.

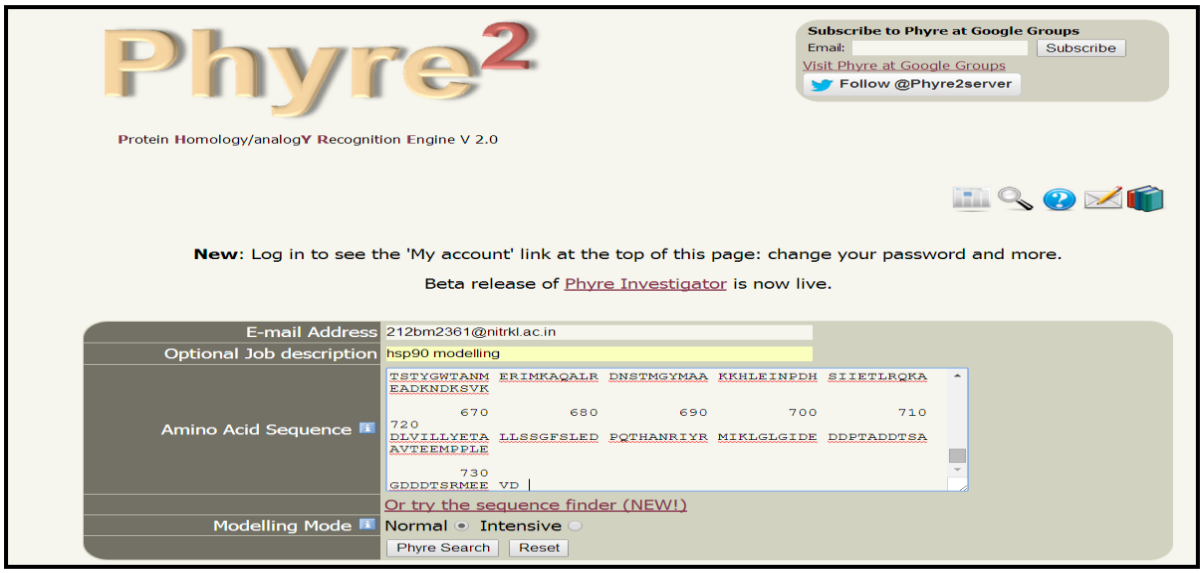


Figure 3:- Webpage of phyre server showing paradigm of modelling enquiries.

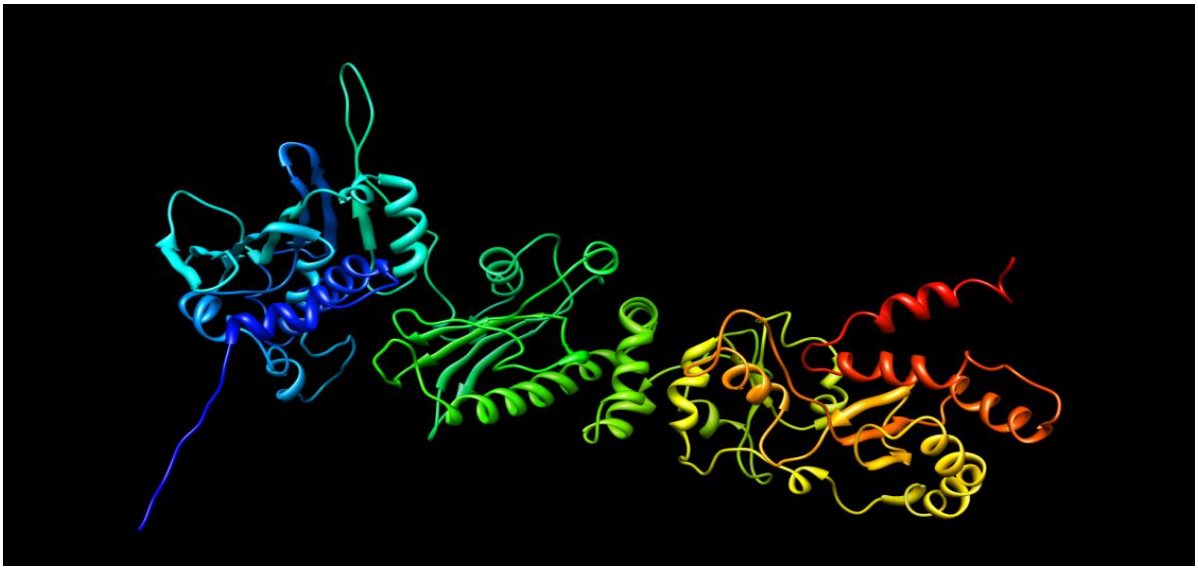


Figure 4:- Full Hsp 90 modelled protein from phyre server

3.4. Retreival of ligands from pubchem database

Pubchem is a free access database containing chemical and structural information of small organic substances, bioassays, patents as well as provide knowledge about their biological activity and cross refer it from original and related structures. PubChem is organized as three linked databases within the NCBI's Entrez information retrieval system.

1. Pubchem with url address https://pubchem.ncbi.nlm.nih.gov/ is browsed.

2. Specific names of Hsp 90 inhibitors are searched.

3. Then specific selection of the type of compound and download the 3D structure of compound in .sdf format.

3.5 Conversion of format of ligand from .sdf to pdb format by using OPEN BABEL

Download Open babel software from internet.

1. The input format was selected as .sdf and the file was browsed from its specific location.

2. The output format of the file was selected as .pdb and convert button was pressed.

3. The output files were generated and saved in the selected folder in .pdb format.

3.6 Designing of hybrid compounds by using Chemsketch

ACD/ChemSketch freeware is a chemically intelligent drawing interface that allows to draw almost any chemical structure including organics, organometallics, polymers, and Markush structures. Draw and view structures in 2D, or render in 3D to view from any angle. It also generate structures from InChI and SMILES strings and IUPAC systematic names for molecules of up to 50 atoms and 3 ring structures. Search for structures in the built-in dictionary of over 165,000 systematic, trivial, and trade names. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P*.

For designing of the hybrid structures, first their structural related chemical properties were studied and then the functional groups of the every drug was taken into account and hybrid

drug was made based on the scaffold of the existing drugs structure. For designing of the structure we have taken into consideration various bonds and functional groups based on their respective property to be laid on to increase the effectiveness of the drug like integration of polar groups like alcohol, amine, amide, carboxylic acid, oxyacid groups which would either ionize or are capable of relatively intermolecular force of attraction and changing the number of methylene groups simulteaneoulsy there leads to increase in lipophilicity or decrease of water solubility and so is the change in degree of unsaturation brings change in rigidity, sensitivity and toxicity. Introductions of groups like halogens and hydroxyl groups would lead to binding site characterization of hydrophilicity character so would lead to change in bonds stability and reactivity of the molecule. Bonds like ester, amide, phosphate and glycosidic bonds bring liability of easy metabolization from various enzymes and lipases.

1. Open ACD/chemsketch software.
2. Import the chemical 2D structure of dimer of Hsp90 inhibitors.
3. Amalgamate two structures by using linker group in between them which could be either any alkyl or acyl bond or could be bond containing ester, amide, phosphate, sulphate or glycosidic groups and further to add or to decrease the properties of the complex either hydrophilic or hydrophobic groups could also be added like apolar groups methyl, acyl and polar groups hydroxyl, halogens respectively.
4. Save the file in .sdf format .

3.7 Docking by using Glide 6.2 tool of Schrodinger software

Protein Preparation:-

Protein preparation wizard of maestro 9.6 consist of 3 steps for preparation of protein

1. **Preprocessing**:- Assigning bond orders, adding hydrogen, creating disulphide bonds, deleting water molecules

2. **Review and modifying**:- After ensuring chemical correctness, hydrogen was added where hydrogen atoms were missing. Side chains that are not close to

the binding cavity and do not participate in salt bridges were neutralized group.

3. **Refining**:- Bond orders and ionization states are properly assigned and performs better when side chains are reoriented when necessary and steric clashes are relieved. The hydrogen bonding network was optimized by reorienting hydroxyl groups, water molecules, and amide groups of Asn and Gln, and selecting appropriate states and orientations of the imidazole ring in His residues.

Optimizing the orientation of the various groups is an iterative process, which passes over all the groups whose H-bonds need to be optimized multiple times. The refinement component performs a restrained impact minimization of the co-crystallized complex. This helps in reorientation of side-chain hydroxyl It uses the OPLS-AA force field (Jorgensen et al., 1996) for this purpose.

1. Import complete protein structure of Hsp 90 consisting of all three domains is prepared from phyre server after taking the fasta sequence from uniprot database.
2. Preprocess the protein structures and remove nonessential water molecules and other ions present in it.
3. Review and modify to check if there is any task undone needed for preprocessing.
4. Finally refine the protein structure by optimizing and then minimizing the restrained structure of Hsp 90.

3.7.2 Active site prediction

To explore the active sites in our selected PDB complex , we have made protein underwent Site map program which generated sufficient information describing the binding site novelties ,it identifies the site by use of grid points and then the contour maps are generated producing the hydrophobic and hydrophilic maps and later accessment of each site is done by calculating various site points ,site size, enclosure and exposure etc and the best binding site is selected with average SiteScore around 1.0.

3.7.3 Ligand preparation

Then these structural formations are assigned an appropriate bond order and optimized in OPLS force field using a default setting (Hayes et al., 2004) using the ligprep script shipped by Schrodinger and finally minimized it low and stabilized energy in 3D format.

The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures. The simplest use of LigPrep produces a single low-energy 3D structure with correct chiralities for each successfully processed input structure and can also produce a number of structures from each input structure with various ionization states.

1. Import all the hybrid molecules in the project table provided in workspace of maestro wizard.
2. Open ligprep script shipped by Schrodinger and browse the selected entries of the ligands.
3. OPLS force field generated ligands were created.

3.7.4 Receptor grid generation

For receptors that adopt more than one conformation on binding, it is necessary to prepare grids for each conformation to ensure that possible actives are not missed. Grid files represent physical properties of a volume of the receptor (specifically the active site) that are searched when attempting to dock a ligand. Also the shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Grids were generated by Receptor Grid Generation panel which defines receptor structure by excluding any co-crystallized ligand that may be present, determines the position and size of the active site as it will be represented by receptor grids, and sets up Glide constraints. Grids were defined by centering them on the ligand in the crystal structure using the default box size.

1. Open receptor grid panel of glide
2. Define the receptor present in workspace
3. Scaling of charge and per atom vander walls radii.
4. Define the grid scaling for providing the binding site to attach the ligand

5. Allow the formation of grid cage near the active site after running the job.

3.7.5 GLIDE docking of inhibitors and ligands

Docking refers to the computational investigation for optimal positioning of a ligand molecule with respect to the binding site of a target structure. We used the docking program GLIDE from Schrodinger which evolves the full span of speed and accuracy from high-throughput virtual screening of several compounds. Docking studies were performed of various designed hybrid drugs on most eminent N-Terminal domain of Hsp90 as it contains the principal ATP-binding site of the chaperone and shows a two-layer sandwich fold thus providing preferable strong binding pocket to the ligands. For interaction of protein and ligand we have used modelled full Hsp90 protein from phyre server. In glide software they have used OPLSAA algorithm which approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor binding pocket using a series of hierarchical filters. XP method is preferred as it weeds out false positives and provide a better correlation between good poses and good scores. Glide Score XP specifically priortizes occupancy of well-defined hydrophobic pockets by hydrophobic ligand groups and also improvizes the scoring of hydrogen bonds as well as detection of buried polar groups and provide accessibility to detection of pi-cation and pi-pi stacking interactions. The XP paradigm for flexible docking includes ligand flexibility in the provided grid box of 50 Å. In flexible docking ligand is centralised at the midpoint between the two most widely separated atoms of the core region of Hsp 90. All 28 ligands were flexible docked with negative XP G Score and the compounds were ranked by the interaction energy.

1. Select the receptor file present in .mae format and specify the type of docking mode to be carried out (XP,SP etc.)
2. Select the file of all ligands which was prepared by using ligprep also present in .mae format.
3. Start docking program after providing the job name.

3.7.6 Ligand interaction analyses to interpret the residues and bonds involved in binding at the binding site

1. Select the docked protein and the specific ligand.
2. Press the ligand interaction icon present in the tool bar of Maestro wizard 9.6.
3. Analyse the interaction of protein residues with ligand molecules as well as the bonds integrating with it like hydrogen, hydrophobic bond, covalent bond etc.

3.8 ADMET /TOX prediction by using Qikprop3.9 script of Schrodinger software

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. QikProp also flags 28 types of reactive functional groups that may cause false positives in high-throughput screening (HTS) assays. It also evaluates the acceptability of analogs based on Lipinski's rule of five (Lipinski et al., 2001., Lipinski et al., 1997), which is essential to ensure drug-like pharmacokinetic profile while using rational drug design. All the analogs were neutralized before being used by Qikprop.

The preferable properties which were taken into consideration for evaluation of the process:-

1. Molecular weight (mol_MW) for better drug likeliness its value must be under (150– 650).
2. Octanol/water partition coefficient (Log Po/w) which denotes for hydrophilicity of both compounds. It has been suggested that high log P value is associated with poor absorption or permeation and it must be within the range of (-2–6.5).

3. Aqueous solubility (QPlogS) which significantly affects its absorption and distribution characteristics. The predicted log S values of the studied compounds were within the acceptable limit of (-6.5–0.5).

4. Apparent MDCK cell permeability (QPPMDCK) is the parameters which mimics the blood brain barrier mandatory for inhibition of cancer their value within 25 depicts poor property where as greater than 500shows elevated property.

5. Brain/blood partition coefficient (QPlogBB) is referred in order to denote the ability of drug to effectively permeate the drug blood brain barrier and could be orally delivered, therefore their range should be within (-3.0–1.2).

6. Percent human oral absorption (C80% is high, B25%is poor)

CHAPTER 4

Results and Discussion

4. Results and Discussion

4.1 Site map of Hsp 90

The location of the primary binding site on a receptor such as a protein is often known from the structure of a co-crystallized complex. Efforts to design better ligands for these receptors can profit from an understanding of how well the known ligands complement the receptor, and how extension of the ligands into adjacent regions could promote binding. site maps explicitly show the shape and extent of phillic and phobic regions, something a surface-based display cannot do. The most important property generated by SiteMap is an overall SiteScore, which has proven to be effective at identifying known binding sites in co-crystallized complexes. As it could be retrieved from the results that site 3 and 4 showed highest site score with highest hydrophobic cavity thus is viable site for finding active site residue as well as binding other hydrophobic residue. Sites 2 and 5 having site score greater than .08 are capable of distinguishing between drug binding and non drug binding site. Similarly like site score, D score which represent the druggability score which distinguishing "difficult" and "undruggable" targets from "druggable" ones.

Table 1:- Different binding sites been searched in full Hsp 90 modelled protein by "Site map" script of Schrodinger.

No. of site map	Site score	Size	Dscore	Volume	Contact	Phobic	Phillic
Site map 3	1.084	298	1.09567	661.647	1.07369	1.156128	1.034849
Site map 4	1.037	129	1.058623	293.608	1.028453	1.307783	1.007047
Site map 1	1.021	412	0.998315	995.043	0.936868	0.506368	1.164753
Site map 2	0.989	370	0.946359	1081.136	0.918335	0.431626	1.236666
Site map5	0.965413	107	0.985962	262.738	0.920571	0.329739	1.048487

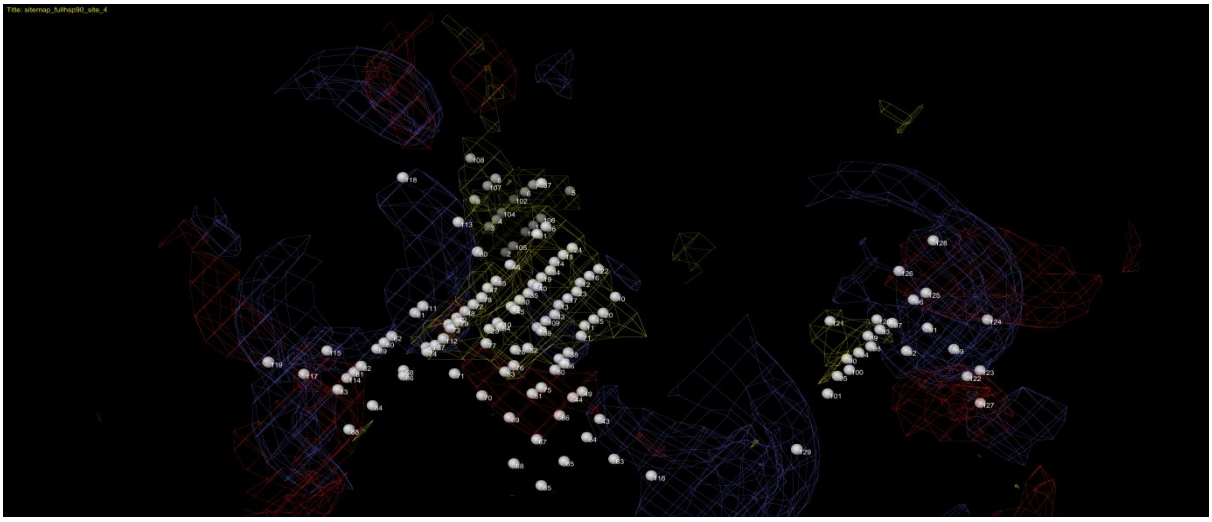


Figure 5:- Site map 3 remarking various nature of residues as under specific property regions (yellow region-hydrophobic, red-hydrohillic and blue-hydrogen bond donor, green-hydrogen bond acceptor).

Later at site 3, from hydrophobic area amino acid residue ASN 51 and Lys 112 are selected as they are also mentioned in binding site residue in uniprot database of Hsp 90 protein selected from human species.

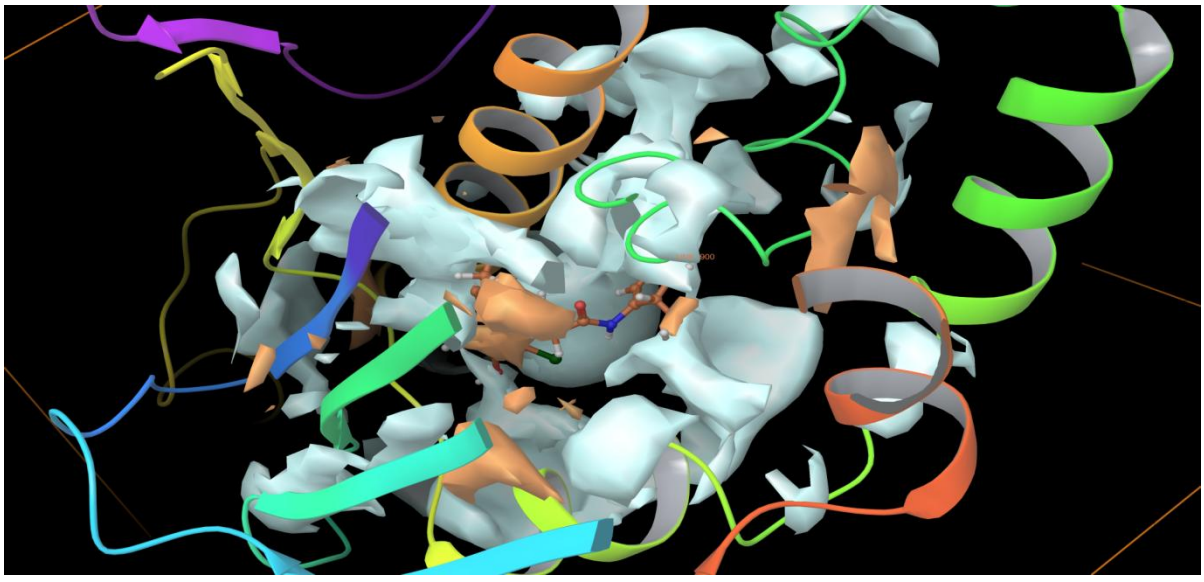


Figure 6:- Depiction of hydrophobhic and hydrophilic map surrounding the resotchol inhibitor.

Van-der Waals and distance-dependent electrostatic-interactions of a probe placed at each of the grid points are then used to generate van-der Waals and electric-field grids. The resultant

van-der Waals and electric-field grids are then used to generate the phobic and philic potentials. Red region is showing phobic region and white region are showing hydrophilic region , Hydrophobic regions that are favourable for occupancy by hydrophobic ligand groups, hydrophilic regions that are favourable for occupancy by hydrophilic ligand groups and neither hydrophobic nor hydrophilic—regions that are of mixed character or are far enough from the receptor surface to be similar to bulk water.

4.3 Hybrid structures formed by chemsketch software

Designing of hybrid structures was based on scaffold of existing drug structures, drug like geldanamycin which was most primiarly drug to inhibit Hsp 90 expression by binding at amino terminal of Hsp 90 by means of ATPase but is also also associated with various demerits like hepatotoxicity, low solubility etc. Although it various analogues have been prepared which has also reached to trial phase but with no successful solution on the contrary radicicol which is also N terminal binding as much greater affinity for binding then geldanamycin and invitro also found to have greater inhibitory activity then GA. Structural Basis for Inhibition of the Hsp90 Molecular Chaperone by the Antitumor Antibiotics Radicicol and Geldanamycin.

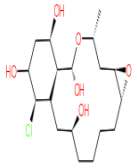
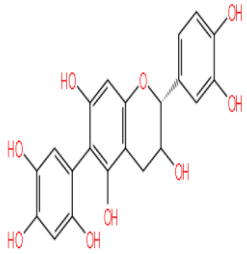
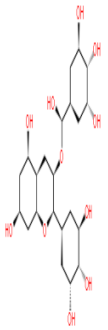
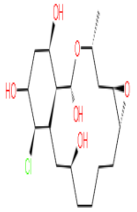
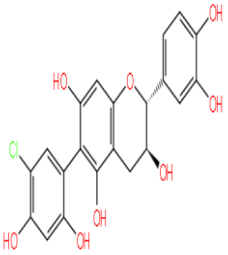
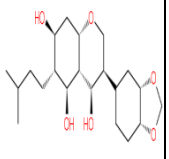
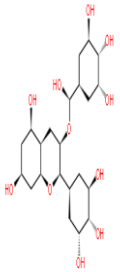
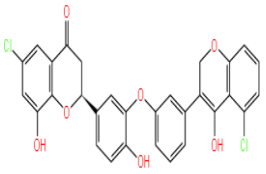
So when chimera complex was formed containing the resorcinol entity of radicicol and quinone entity of geldanamycin, joined by varied linker group and at various sites of drugs .The various compounds which were formed are like H7 are joined by amide linker group, H12 by ester linkage and compounds like H16 are macrocyclic entities. After molecular docking they were concluded to have better binding affinity as they are composed of additive properties of two natural drugs and thus enhanced potency and affinity (Shen et al., 2009).

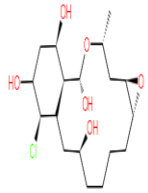
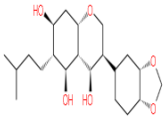
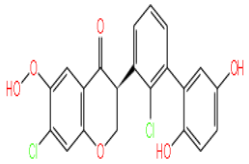
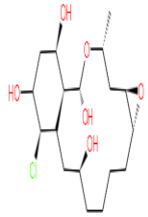
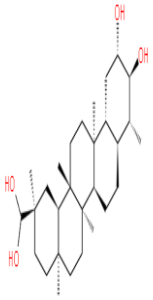
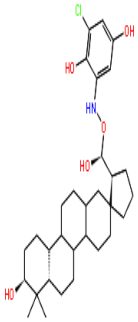
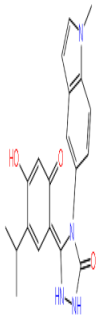
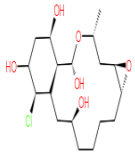
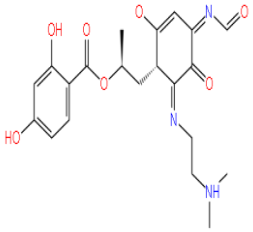
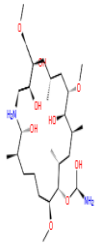
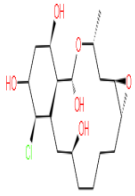
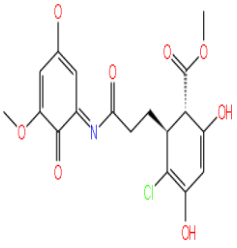
On the same ground of concepts hybrids of other drugs like radicicol nd PU3 are formed which are again desired to have better affinity and combined properties of two drugs.As we are aware of the fact that Hsp 90 ATP binding pocket contains both hydrophobic and hydrophilic pocket (Jeong et al., 2014).With ligand interaction it was revealed that, and radicicol a containing resorcinol group is entities exposed towards the solvent and polar groups like Thr109, Asn 106, Asn 51 where as PU3 functional entity was facing towards hydrophobic residues like Phe 138, Met 96 , Ala 55 etc. So this interaction and amalgamation of functional entities of existing drugs has strengthened our envision of better effective

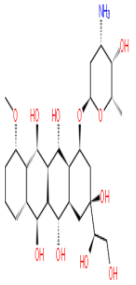
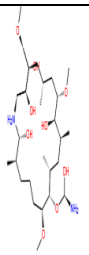
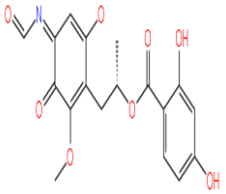
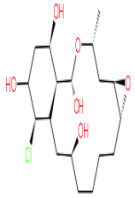
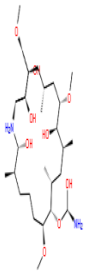
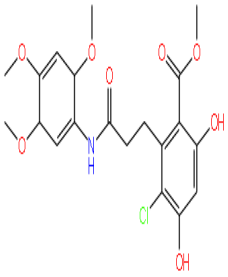
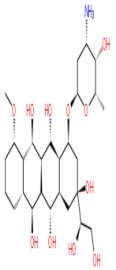
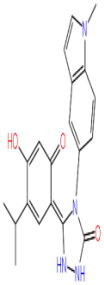
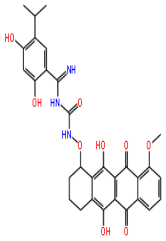
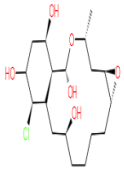
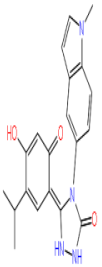
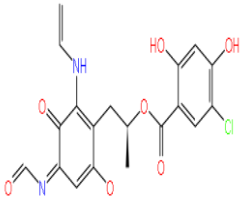
inhibition of Hsp 90. With respect to other perks achieved from hybrid theory they have also deduced to have preferential admet properties also which make them more susceptible towards cancer therapy.

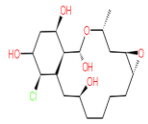
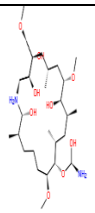
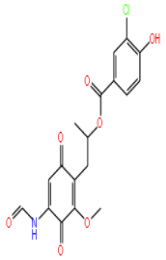
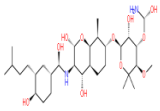
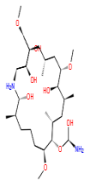
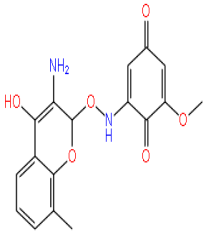
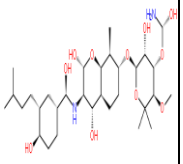
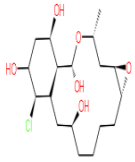
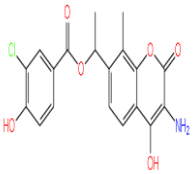
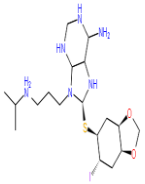
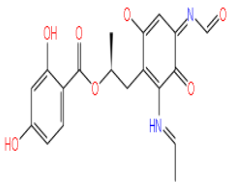
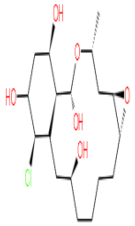
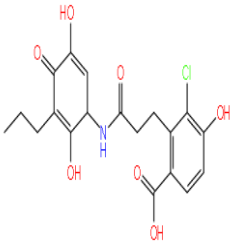
Other significant complexes were formed like between N-Terminal and C-Terminal drugs , as they provide more flexibility towards two binding sites and thus more embarked inhibition of Hsp 90. Novobiocin belonging from the class of flavonoids contains pyrazole rings having 3-dimethylally l-4-hydroxybenzoyl moiety (ring A), a 3-amino-4,7-dihydroxycoumarin moiety (ring B). This compound scaffold is when merged with functional groups of other drugs like quinone group of geldanamycin, resorcinol group of radicicol or chalcone group, then they exhibit improved and cumulative properties of both drugs as aminocoumarin entity is flanging away from the active site and the group near the bonding site is that of resorcinol or quinone as they are covered with many hydrophobic residues as well as showing more intimacies with area near the active residue (Cruz et al., 2010).

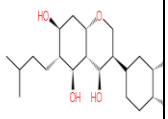
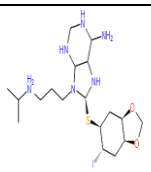
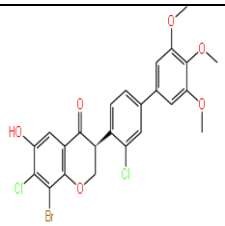
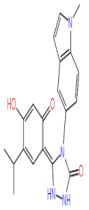
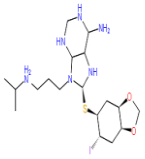
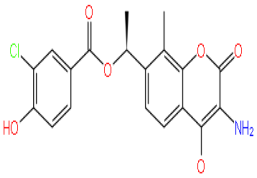
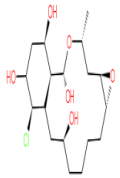
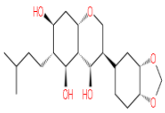
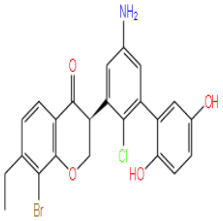
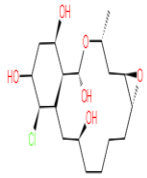
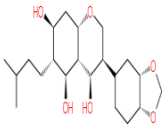
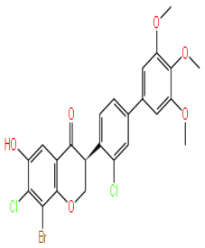
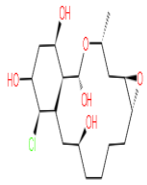
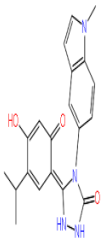
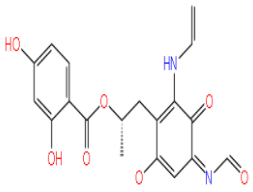
Table 2:- Illustration of structures of existing Hsp 90 inhibitors and from them formed hybrid structures and their respective affinity towards particular terminal.

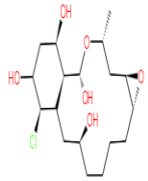
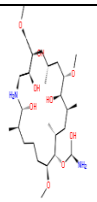
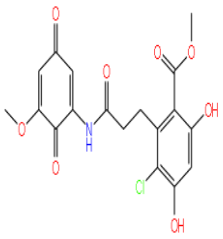
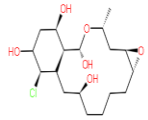
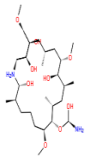
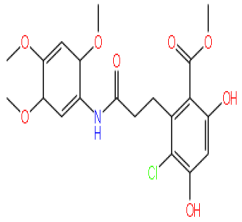
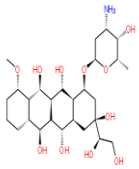
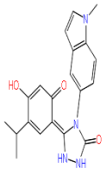
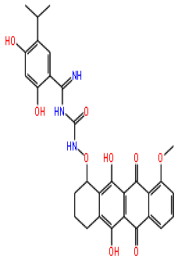
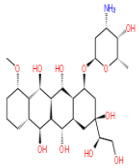
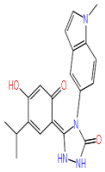
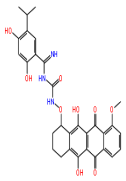
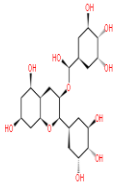
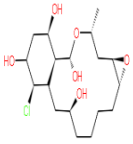
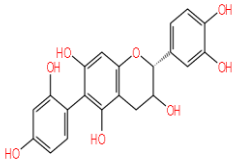
S.No.	Drug 1	Affinity Towards Site	Drug 2	Affinity Towards Site	Hybrid Structure	Affinity towards the ATP binding site
1.	EGCG 	CTD	RADICICOL 	NTD	(RAD-EGCG1)(H1) 	NTD
2.	EGCG 	CTD	RADICICOL 	NTD	(RAD-EGCG)(H2) 	NTD
3.	DERRUBONE 	CTD	EGCG 	CTD	(DERR-EGCG)(H3) 	NTD

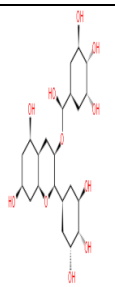
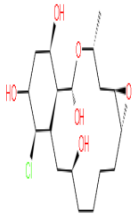
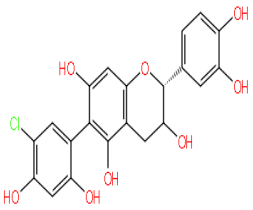
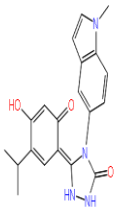
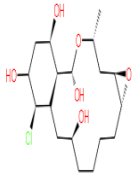
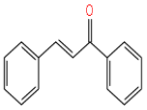
4.	RADICICOL 	NTD	DERRUBONE 	CTD	(DERR-RAD1)(H4) 	NTD
5.	RADICICOL 	NTD	CELASTROL 	CTD	(RAD-CEL)(H5) 	NTD
6.	17DMAG 	NTD	MONOCILIN 	NTD	(17DMAG-MONO) (H6) 	NTD
7.	GELDANAMY CIN 	NTD	RADICICOL 	NTD	(GEL-RAD)(H7) 	NTD
8.	RADICICOL	NTD	GELDANAMY CIN	NTD	(RAD-GEL1)(H8)	NTD

						
9.	RADICICOL 	NTD	GELDANAMYCIN 	NTD	(RAD-GEL 2)(H9) 	NTD
10.	DOXORUBICIN 	CTD	GANATESPIB 	NTD	(DOX-GAN) (H10) 	NTD
11.	RADICICOL 	NTD	17AAG 	NTD	(RAD-17AAG)(H11) 	NTD
12.	RADICICOL	NTD	GELDANAMYCIN	NTD	(RAD-GEL2)(H12)	NTD

						
13.	NOVOBIOCIN 	CTD	GELDANAMYCIN 	NTD	(NOV-GEL)(H13) 	NTD
14.	NOVOBIOCIN 	CTD	RADICICOL 	NTD	(NOV-RAD)(H14) 	NTD
15.	GELDANAMYCIN 	NTD	PU3 	NTD	GEL-PU3 (H15) 	NTD
16.	GELDANAMYCIN 	NTD	RADICICOL 	NTD	(RAD-GEL3)(H16) 	NTD
17.	DERRUBONE	CTD	PU3	NTD	PU3ISOFLAVO(PU3-DERRU)(H17)	NTD

						
18.	VER49009 	NTD	PU3 	NTD	(PU3-VER)(H18) 	NTD
19.	RADICICOL 	NTD	DERRUBONE 	CTD	(DERR-RAD 2)(H19) 	NTD
20.	RADICICOL 	NTD	DERRUBONE 	CTD	(DERR-RAD3)(H20) 	NTD
21.	RADICICOL 	NTD	17AAG 	NTD	(RAD-17AAG(H21) 	NTD
22.	RADICICOL	NTD	GELDANAMY CIN	NTD	(RAD-GEL)(H22)	NTD

						
23.	RADICICOL 	NTD	GELDANAMYCIN 	NTD	(RAD-GEL 2)(H23) 	NTD
24.	DOXORUBICIN 	CTD	GANATESPIB 	NTD	(DOX-GAN)(H24) 	NTD
25.	DOXORUBICIN 	CTD	GANATESPIB 	NTD	(DOX-GAN(H25) 	NTD
26.	EGCG 	CTD	RADICICOL 	NTD	(RAD-EGCG(H26) 	NTD
27.	EGCG	CTD	RADICICOL	NTD	(RAD-EGCG 4) (H27)	NTD

						
28.	VER-49009 	NTD	RADICICOL 	NTD	(VER-RAD1) (H28) 	NTD

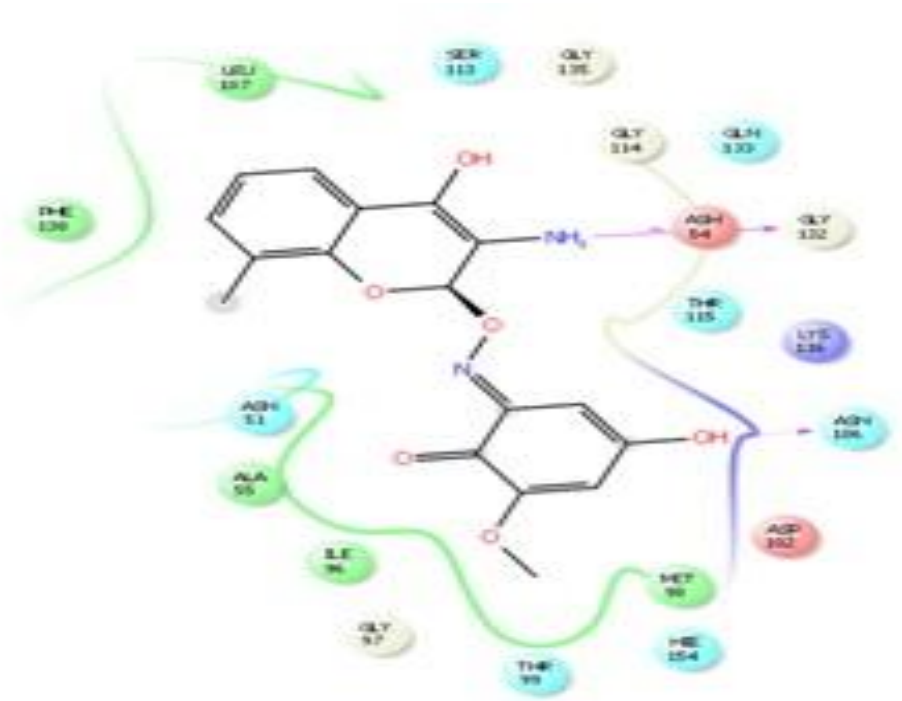


Figure 7:- Ligand interaction diagram showing the interaction of different functional group with amino acid residues of Hsp 90.

Similarly other chimeric compounds were formed containing isoflavone group and catechin group from flavanoids which are the obtained natural from natural source like green tea, grapes, blue berries etc they are the source of pharmaceutical potentials like anti-allergic,

anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal and antiviral), anti-cancer, and anti-diarrheal activities, and led to formation of hybrid with highly potentiated qualities. Anthracycline inhibitors like doxorubicin, epirubicin containing polyketide chain though they are successful CTD Hsp 90 inhibitors but again they are associated with severe side effects like drug induced heart failure, neutropenia, leukopenia, hand and foot syndrome etc which restrict their isolated usage. So combining its structure with enhanced quanlity natural drugs can serve as better option for cancer therapy.

4.4 Docking of hybrid drugs at both domains of Hsp 90

Table 4: Table depicting binding affinity of various existing drugs derived from Glide (Schrodinger software

Hsp0 inhibitor	**Binding affinity at NTD (Docking score)**	**Binding affinity at CTD (Docking score)**
EGCG	-8.420	-6.556
Derrubone	-8.133	-4.191
Doxorubicin	-7.919	-6.542
Novobiocin	-6.620	-5.228
Radicicol	-5.740	-4.134
Celastrol	-5.132	-5.06
Ganatespib	-5.172	-3.052
Epirubicin	-6.564	-5.191
Geldanamycin	-5.175	-5.393
PU 3	-4.699	-3.133
17dmag	-5.578	-5.478
17aag	-5.892	-5.745
Monocillin	-5.689	-4.854

Table 5:- Respective hybrid compound drugs and their source existing inhibitors with their affinity towards specific domain of Hsp90

S.No.	Name	Docking Score at ASN 51 (NTD)	Glide Score	Docking score at LYS 112 (NTD)	Docking score at Lys 632 (CTD)	Prime MGBSA δ BIND
1.	(H1)	-7.946	-7.946	-10.115	-3.814	-62.552
2.	1YET ligand	-7.894	-7.894	-9.457	-5.342	-95.987
3.	(H2)	-7.559	-7.583	8.99597	-3.224	-69.352
4.	(H3)	-7.450	-7.450	8.8854	-2.874	-72.306
5.	(H4)	-6.862	-6.863	8.66995	-2.478	-69.307
6.	(H5)	-6.687	-6.694	7.96083	-2.845	-44.323
7.	(H6)	-6.794	-6.847	7.55376	-3.378	66.228
8.	(H7)	-6.613	-6.905	7.37712	-4.166	60.823
9.	(H8)	-6.420	-6.451	6.8989	-3.618	58.923
10	(H19)	6.453	-6.454	6.76364	-3.654	73.23
11	(H22)	-6.335	-6.640	6.39227	-3.779	-73.330

12	(H9)	-6.341	-6.558	6.23161	-4.60892	-76.581
13	(H23)	-6.081	-7.339	6.10123	-4.30851	-65.780
14	(H24)	-6.300	-6.389	6.0312	-4.25435	63.3307
15	(H21)	-6.136	-6.178	6.00353	-3.37114	53.0832
16	(H25)	-6.088	-7.880	5.94137	-4.09472	76.3303
17	(H10)	-5.870	-5.960	5.91688	-3.89678	50.4574
18	(H17)	-5.805	-6.174	-5.689	-2.78604	64.6302
19	(H20)	5.744	-5.745	-5.819	-2.568	-68.958
20	(H12)	5.565	5.725	5.82252	-2.487	-65.594
21	(H26)	-5.080	-5.103	5.81752	-2.92873	-50.934
22	(H13)	-5.074	-5.577	5.21148	-2.5364	-81.267
23	(H14)	-4.886	-5.206	-5.07334	-2.30372	-49.533
24	(H15)	-4.604	-4.902	-3.247	-5.697	-82.722

25	(H16)	-4.553	4.566	-3.854	-4.20606	-26.530
26	(H11)	3.033	-3.034	-4.086	-2.369	-74.532
27	(H17)	1.834	-2.385	-3.818	-3.458	-67.267
28	(H28)	-3.658	-3.658	-3.213	-2.874	51.658
29	(H27)	.0156	-2.255	-3.053	-2.928	-44.982

Table 6:Final representation of promising hybrid drugs having better binding affinity from their derived drugs.

Hybrid Drug name	**Parent Drugs docking score**		**Hybrid drug score**	**Binding site**
	Drug 1	**Drug 2**		
H1(EGCG-RAD)	-8.420	-5.740	-10.115	NTD
H2(EGCG-RAD)	-8.420	-5.740	-8.995	NTD
H3(EGCG-DOC)	-8.420	-7.919	-8.885	NTD
H4(RAD-DOC)	-5.740	-7.919	-8.669	NTD
H5(CEL-RAD)	-5.132	-5.740	-7.960	NTD
H6(17DMAG-MONO)	-5.175	-4.120	-7.553	NTD

In all the drugs, H1 showed highest binding affinity as its hydroxyl groups are attached with charged negative Asp 102 by hydrogen bonding and hydroxyl groups are interacting with charged positive Lys 116 bonded with hydrogen bonding in the hydrophobic vicinity of HSP 90.Additionally this group is also surrounded by polar Glu 133, Asn 51,Leu 96,Ala 55 and Met 98 providing it the hydrophillic arena.

H1 is hybrid of two functional groups resorcinol and catechin which is derived from natural source of green tea leaves and is powerful antioxidant and anticarcinogenic agent whereas resorcinol is derived from resins and is also nice antiseptic and resorcinol moiety binds in the same location as adenine ring of ADP and mimics the hydrogen bond donor /acceptor properties of exo and N7 endocyclic amine /imin respectively and when collectively docked to Hsp 90 N Terminal domain ,resorcinol moiety showed greater affinity towards the N Terminal and got attached near Asn 51 and Lys 112 with catechin group having more phillicity towards C Terminal and it was hanging backwards.

The docked poses were then minimized using the local optimization feature in Prime, and the energies were calculated using the OPLS-AA force field and the GBSA continuum model in Maestro. The binding free energy ΔGbind is estimated as where ΔEMM is the difference in energy between the complex structure and the sum of the energies of the ligand and unliganded protein, using the OPLS force field, ΔGsolv is the difference in the GBSA solvation energy of the complex and the sum of the solvation energies for the ligand and unliganded protein, and ΔGSA is the difference in the surface area energy for the complex and the sum of the surface area energies for the ligand and uncomplexed protein.

$$\Delta G\text{bind} = \Delta E\text{MM} + \Delta G\text{solv} + \Delta G\text{SA} \quad \text{.....(1)}$$

Higher the prime energy better is the values of the stability of the conformer.

4.5 ADMET/TOX Study

All the structures showed significant values for the properties analyzed (Table 7) and showed drug-like characteristics based on Lipinski's rule of 5.The first three properties are based on Lipinski rule of five, molecular weight (mol_MW) less than 650, partition coefficient between octanol and coefficient between octanol and water (logPo/w) between -2 and 6.5 and solubility (QPlogS) greater than -7. Brain/blood partition coefficient (QPlogBB) parameter indicated about the ability of the drug to pass through the blood–brain barrier which is mandatory for inhibition of cancer. Whereas QPPMDCK predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier. Higher the value of MDCK cell, higher the cell permeability. All designed compounds had showed ADME properties in acceptable range. On taking detailed look we found that drug H1 showed lowest molecular weight thus low dose uptake and has higher

water solubility in reference to geldanamycin, similarly its efficiency to cross blood brain barrier is also sufficient enough with high percentage of human oral absorption providing it more impetus over other drugs as it contains highest binding affinity thus more affirmation in inhibiting Hsp 90. In view of other drugs like H2 and H3, these drugs also have comparatively similar properties to geldanamycin in context of water solubility, brain membrane permeability,oral absorption but due to their greater weight index they are having liability of heavier dose thus greater toxicity.

Table 7:-**Depiction of various admet properties of each hybrid compound.**

Drug Name	**Mol. wt (Molecular weight)**	**QP Log S (Solubility coefficient)**	**QP Log Po/V(water/octanol coefficient)**	**QP LOG BB(Blood brain barrier coefficient)**	**QPLOGMMDCK (cell memebrane permeability coefficient)**	**%Human Absorption**
(H1)	398.36	-3.556	0.76	-2.903	5.416	39.662
(H2)	432.81	-4.166	1.254	-2.717	14.987	43.82
(H6)	431.44	-2.085	0.775	-2.48	2.813	47.272
(H7)	413.81	-3.327	1.08	-2.176	41.864	64.685
(H8)	375.33	-3.379	0.847	-2.908	7.805	55.759
(H11)	413.81	-3.225	0.85	-2.35	24.904	62.152
(H10)	409.77	-3.809	1.104	-2.867	12.61	56.645
(H15)	575.57	-4.978	2.566	-3.211	4.293	22.653
(H14)	386.36	-3.389	0.906	-3.019	6.23	54.482
(H15)	575.57	-6.705	2.648	-4.251	2.075	14.468
(H16)	575.57	-6.293	3.01	-3.491	6.257	27.962
(H17)	420.80	-3.982	1.36	-2.815	19.238	59.14
(H13)	413.81	-3.335	0.832	-2.597	12.968	57.232
(H10)	411.79	-3.308	1.433	-2.155	40.427	66.965
(H11)	420.80	-3.912	1.411	-2.734	20.569	59.939
(H20)	432.81	-4.181	1.217	-2.782	12.829	42.439
(H21)	344.32	-2.737	0.521	-1.882	31.326	63.848
(H22)	575.57	-6.52	2.634	-4.142	2.381	15.13
(H28)	432.81	-4.214	1.251	-2.762	13.898	43.212
(H22)	389.79	-3.77	1.376	-2.004	25.178	61.178
(H25)	563.39	-8.157	5.793	-0.944	1042.2	83.047
(H4)	488.76	-6.184	3.794	-1.387	245.3	89.507
(H5)	548.16	-6.759	4.617	-1.383	321.05	85.516
(H9)	433.24	-4.834	2.762	-1.341	192.72	81.683
(H18)	415.27	-6.928	4.68	-0.622	1490.1	100

(H26) (H29) (H23)	554.22 422.86	-7.979 -5.798	5.914 3.722	-0.128 -0.807	9507.9 454.37	93.952 100

Most of drug candidates fail in clinical trials due to poor ADME properties. Thus, an important aspect of drug discovery is to avoid compounds not having drug likeliness and good ADME property. So to streamline the virtual screening, drug likeliness and ADME properties of all the thirty compounds were predicted using QikProp, version 3.4 of Schrodinger 2013.Lipinski filter and reactive filter were applied before virtual screening to avoid false positive lead molecule using Qik prop script of schrodinger (Meraj et al., 2013). Lipinski filter rejected ligands not following Lipinski rule of five and reactive filter rejected ligands with reactive functional groups and in case non-violation of rule shoed the value of zero. Drug likeliness, log P, log S, molecular weight and toxicity risks may be used to judge the compound's overall potential to qualify a ligand as potential drug candidate.

All twenty eight ligands have appropriate logP (octanol/water) value for biological efficacy. Each of them had zero Lipinski violation and satisfying pharmacological properties of 95% available drugs with high to medium predicted oral absorption availability. Molecular weight of most of the ligand falls within the range of 297-404 Daltons. The ligands are having no toxic functional groups. Log S values of these ligands are within the acceptable range of 95% of existing drugs. The overall pharmacological properties of these ligands justify that the molecules are biologically active without any toxic functional groups. Hydrophobic compounds have relatively poor solubility, high log P, and high serum protein binding, but good cell permeability; whereas the opposite is true for hydrophilic compounds.

This dichotomy was responsible for the classic lead optimization struggle of solubility versus permeability. Poor oral availability and permeability may lead to drug failure. The four lead molecules like H1,H2 and H3 reported in the present study are well within the hydrophobic and hydrophilic extremes at the same time percentage of oral availability is also high.

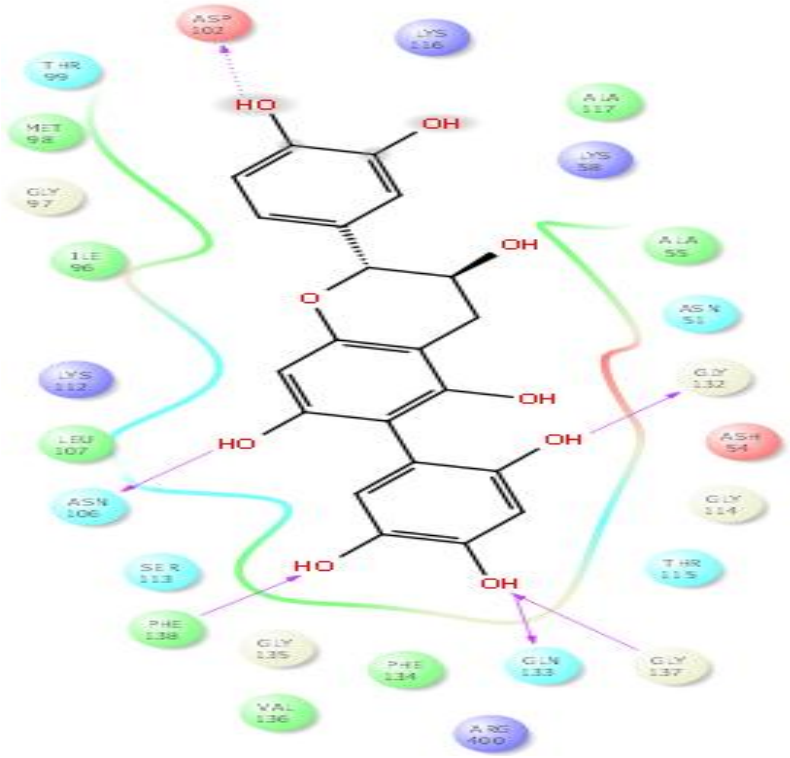


Figure 8:- Plot of ligand (resotchol) interaction with HSP 90(Ligand interaction tool, Maestro 9.6,Schrodinger)

4.5 Molecular surface representation of Hsp90 bonded with ligand

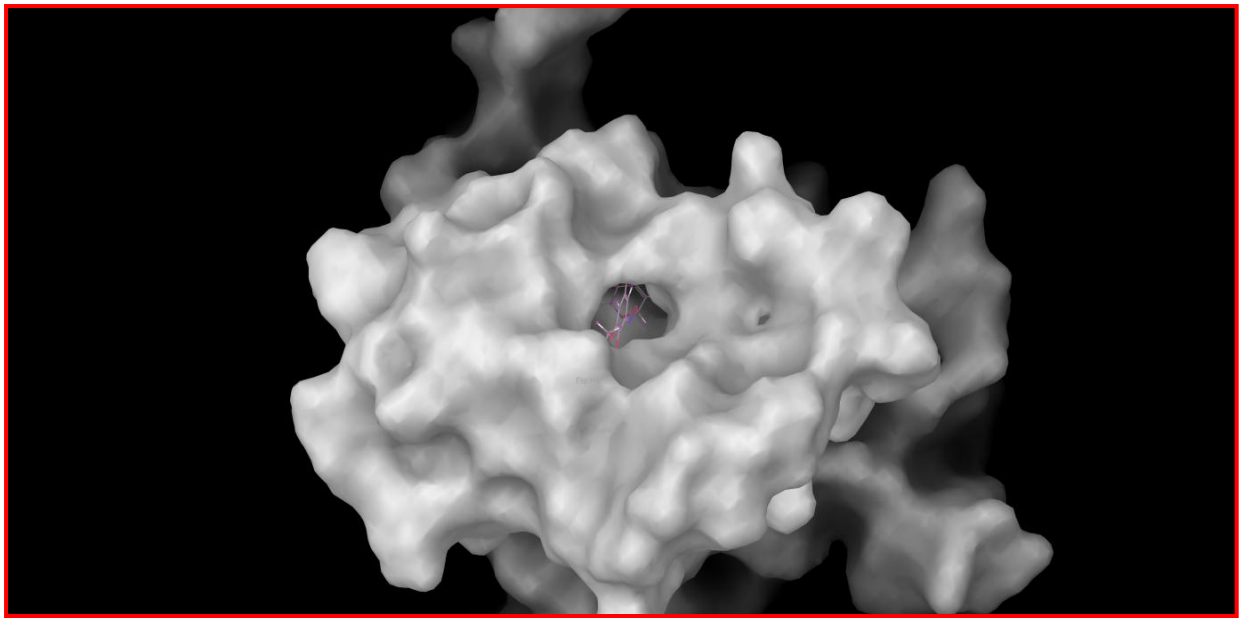


Figure 9:- This figure shows cavity inside the receptor molecule for perfect fitting of the ligand molecule into the Hsp 90 protein structure (Schrodinger,Maestro 9.6).

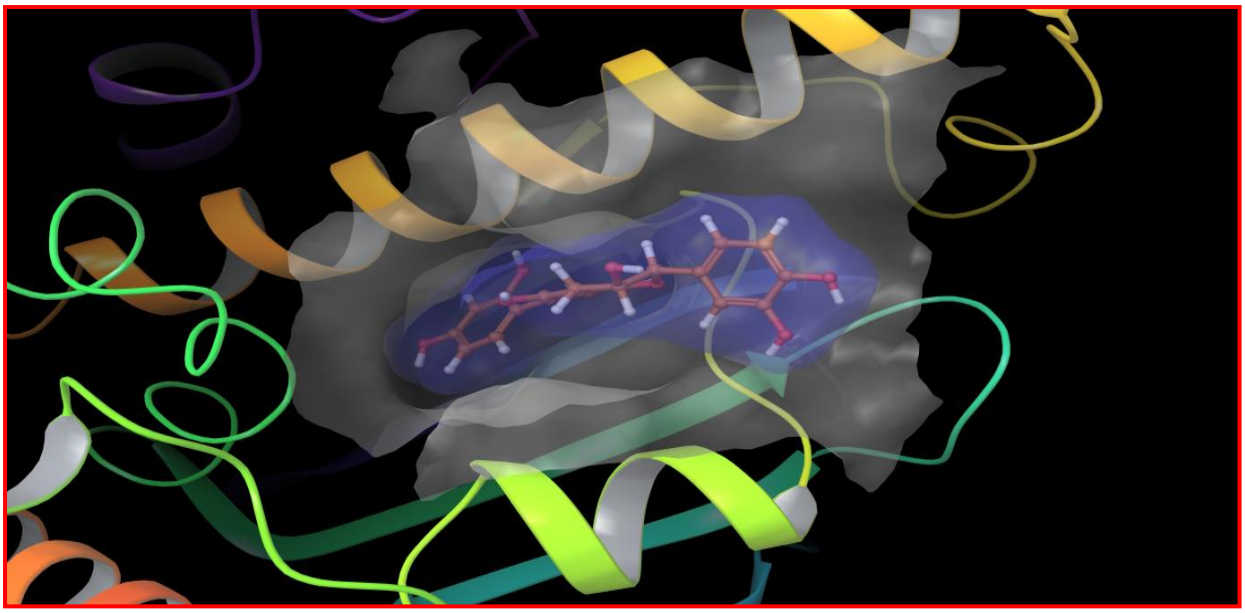


Figure 10:-Picture depicting the pocket of Hsp 90 grasping resotichol (Description:- Surface molecular mesh created for the particular ligand to represent the part of surface volume participating of Hsp 90 protein in docking with the desired ligand).

CHAPTER 5

Conclusion and Future Prospectives

5.1. Conclusion

Protein ligand interaction is one of the major mechanisms controlling various paradigms of signalling and stabilization networking. Heat shock protein 90 is rational target and important molecular chaperone protein having essentiality in stabilization for continued function of overexpressed, hybrid or multiple mutated proteins which could turn out to be carcinogenic. To inhibit its normal functioning it is made to interact with inhibitors whose effectiveness depends on their binding affinity with Hsp 90 protein. We have hypothesized new avenue in field of Hsp 90 inhibitors by designing hybrid inhibitors based on the scaffold of existing drugs. The basic chemical structures of existing drugs like benzoquinone of geldanamycin, resorcinol of radicicol was retrieved and made to ligate with each other by using various linker groups. About 30 hybrid structures were designed by using chemsketch tool, which were made to undergo stereo chemical stabilization and energy minimization. Further to validate their drug ability they were checked by Lipinski rule of five and rule of three.

To predict the active site for interaction of ligand ,site mapping tool of schrodinger was used which predicted five major site locations in which site 3 was assumed to have maximum probability of having most favourable active site as it had highest site score of 1.084 and D score of value 1.095. After site prediction, when all hybrid drugs were docked to both the domains of Hsp 90, then in N terminal domain, most of hybrid drugs secured maximum binding affinity in comparison to C terminal.

After comparative analysis of existing drugs and hybrid drugs on inhibitory potential, we have found out that our hybrid drugs attained better glide score. Among 28 structures which were docked to Hsp 90 protein at active site Lys 112 of N terminal, five leading hybrids H1,H2,H3 ,H4 and H5 were selected as they secured maximum glide score of 10.115, 9.457, -8.995, 8.885 and 8.669 respectively. We have investigated that in all the leading hybrid compounds existing drugs like EGCG, radicicol and geldanamycin were most common participants in drug designing as well as they also showed individual maximum glide score .So it was also concluded that groups like catechin of EGCG ,resorcinol of geldanamycin and benzoquinone of geldanamycin have most energetically and stereochemically favourable interaction with Hsp 90 protein.

Further in order to investigate the pharmacological dynamics of hybrid ligands and protein, ADMET prediction was done by using Qik Prop tool of Schrodinger. Then it

was analysed that drug H1 showed most favourable pharmacological profile with lowest molecular weight thus probability of low dose consumption and better QPMDDCK,QP logBB value better from their existing drugs ,thus have less chances of side effects and lower toxicity values. So in an attempt to find out the amenable hybrid inhibitor by insilico methodology, we have investigated that hybrid drugs showed better molecular interaction as indicated by glide score and also have probability to show better and satisfying pharmacokinetic profile and thus could lead into promising future drug for unveiling the HSP 90 structure activity relationship and thus combating the dreadful disease of cancer.

5.2 Future perspectives

Further in order to explore the pharmacophore profiling and to better understand the structure activity relationship, 3D QSAR modelling could be done in order to synchronise the physical modelling of protein and its biological activities. Progressively we could also do molecular dynamics simulation in order to make most realistic in–vivo model for more assured understanding of protein ligand as well as protein–protein interaction.

REFERENCES

1. Akbar H, Cardoso FC, Meier S, Burke C, McDougall S, Mitchell M. Postpartal Subclinical Endometritis Alters Transcriptome Profiles in Liver and Adipose Tissue of Dairy Cows. Bioinformatics and biology insights 2014; 8:45.
2. Altreuter DH, Dordick JS, & Clark DS. Nonaqueous biocatalytic synthesis of new cytotoxic doxorubicin derivatives: exploiting unexpected differences in the regioselectivity of salt-activated and solubilized subtilisin. Journal of the American Chemical Society 2002; 124(9):1871-1876.
3. Anderle C, Stieger M, Burrell M, Reinelt S, Maxwell A, Page M. Biological activities of novel gyrase inhibitors of the aminocoumarin class. Antimicrobial agents and chemotherapy 2008; 52(6):1982-1990.
4. Binder RJ, Blachere NE, & Srivastava PK. Heat shock protein-chaperoned peptides but not free peptides introduced into the cytosol are presented efficiently by major histocompatibility complex I molecules. Journal of Biological Chemistry 2001; 276(20):17163-17171.
5. Bongarzone S & Bolognesi ML. The concept of privileged structures in rational drug design: focus on acridine and quinoline scaffolds in neurodegenerative and protozoan diseases. Expert opinion on drug discovery 2011; 6(3):251-268.
6. Bouma J, Beijnen J, Bult A, & Underberg W. Anthracycline antitumour agents. Pharmaceutisch Weekblad 1986; 8(2):109-133.
7. Budillon A, Di Gennaro E, Bruzzese F, Rocco M, Manzo G, & Caraglia M. Histone deacetylase inhibitors: a new wave of molecular targeted anticancer agents. Recent patents on anti-cancer drug discovery 2007; 2(2):119-134.
8. Chahar MK, Sharma N, Dobhal MP, & Joshi YC. Flavonoids: A versatile source of anticancer drugs. Pharmacognosy reviews 2011; 5(9):1.
9. Chiosis G, Huezo H, Rosen N, Mimnaugh E, Whitesell L, & Neckers L. 17AAG: Low Target Binding Affinity and Potent Cell Activity—Finding an Explanation1. Molecular Cancer Therapeutics 2003; 2(2):123-129.
10. Clevenger RC, Raibel JM, Peck AM, & Blagg BS. Biotinylated geldanamycin. The Journal of organic chemistry 2004; 69(13):4375-4380.
11. Donnelly A & Blagg BS. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. Current medicinal chemistry 2008; 15(26):2702.

12. Du G-J, Zhang Z, Wen X-D, Yu C, Calway T, Yuan C-S. Epigallocatechin Gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. Nutrients 2012; 4(11):1679-1691.
13. Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. Cancer research 2008; 68(8):2850-2860.
14. Gianni L, Herman EH, Lipshultz SE, Minotti G, Sarvazyan N, & Sawyer DB. Anthracycline cardiotoxicity: from bench to bedside. Journal of Clinical Oncology 2008; 26(22):3777-3784.
15. Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. Journal of Biological Chemistry 1997; 272(38):23843-23850.
16. Hastings JM, Hadden MK, & Blagg BS. Synthesis and evaluation of derrubone and select analogues. The Journal of organic chemistry 2008; 73(2):369-373.
17. Heide L. New aminocoumarin antibiotics as gyrase inhibitors. International Journal of Medical Microbiology 2013.
18. Hummel J, Segu S, Li Y, Irgang S, Jueppner J, & Giavalisco P. Ultra performance liquid chromatography and high resolution mass spectrometry for the analysis of plant lipids. Frontiers in plant science 2011; 2.
19. Hunter MC, O'Hagan KL, Kenyon A, Dhanani KC, Prinsloo E, & Edkins AL. Hsp90 Binds Directly to Fibronectin (FN) and Inhibition Reduces the Extracellular Fibronectin Matrix in Breast Cancer Cells. PloS one 2014; 9(1):e86842.
20. Ikuina Y, Amishiro N, Miyata M, Narumi H, Ogawa H, Akiyama T. Synthesis and antitumor activity of novel O-carbamoylmethyloxime derivatives of radicicol. Journal of medicinal chemistry 2003; 46(12):2534-2541.
21. Jia J, Xu X, Liu F, Guo X, Zhang M, Lu M. Identification, Design and Bio-Evaluation of Novel Hsp90 Inhibitors by Ligand-Based Virtual Screening. PloS one 2013; 8(4):e59315.
22. Jolly C & Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. Journal of the National Cancer Institute 2000; 92(19):1564-1572.

23. Kapetanovic I. Computer-aided drug discovery and development (CADDD):< i> In silico</i>-chemico-biological approach. Chemico-biological interactions 2008; 171(2):165-176.
24. Meraj K, Mahto MK, Christina NB, Desai N, Shahbazi S, & Bhaskar M. Molecular modeling, docking and ADMET studies towards development of novel Disopyramide analogs for potential inhibition of human voltage gated sodium channel proteins. Bioinformation 2012; 8(23):1139.
25. Moulin E (2006) Diversity-oriented Synthesis of Pochonins: A Privilege Scaffold for ATPase and Kinase Inhibition. (Strasbourg 1).
26. MR Patlolla J & V Rao C. Triterpenoids for cancer prevention and treatment: current status and future prospects. Current pharmaceutical biotechnology 2012; 13(1):147-155.
27. Neckers L (2007) Heat shock protein 90: the cancer chaperone. Heat Shock Proteins in Cancer, (Springer), pp 231-252.
28. Padhye S, Yang H, Jamadar A, Cui QC, Chavan D, Dominiak K. New difluoro Knoevenagel condensates of curcumin, their Schiff bases and copper complexes as proteasome inhibitors and apoptosis inducers in cancer cells. Pharmaceutical research 2009; 26(8):1874-1880.
29. Park H, Kim Y-J, & Hahn J-S. A novel class of Hsp90 inhibitors isolated by structure-based virtual screening. Bioorganic & medicinal chemistry letters 2007; 17(22):6345-6349.
30. Peck B, Chen C-Y, Ho K-K, Di Fruscia P, Myatt SS, Coombes RC. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. Molecular Cancer Therapeutics 2010; 9(4):844-855.
31. Peng B, Xu L, Cao F, Wei T, Yang C, Uzan G. Research HSP90 inhibitor, celastrol, arrests human monocytic leukemia cell U937 at G0/G1 in thiol-containing agents reversible way. Mol Cancer 2010; 9:79.
32. Pérez-Cruz F, Vazquez-Rodriguez S, Matos MJ, Herrera-Morales A, Villamena FA, Das A. Synthesis and Electrochemical and Biological Studies of Novel Coumarin–Chalcone Hybrid Compounds. Journal of medicinal chemistry 2013; 56(15):6136-6145.
33. Pratt WB, Morishima Y, Peng H-M, & Osawa Y. Proposal for a role of the Hsp90/Hsp70-based chaperone machinery in making triage decisions when proteins

undergo oxidative and toxic damage. Experimental Biology and Medicine 2010; 235(3):278-289.

34. Saadeh HA, Mosleh IM, & Mubarak MS. Synthesis of novel hybrid molecules from precursors with known antiparasitic activity. Molecules 2009; 14(4):1483-1494.
35. Soares P, Dias S, Novo C, Ferreira I, & Borges J. Doxorubicin vs. ladirubicin: methods for improving osteosarcoma treatment. Mini reviews in medicinal chemistry 2012; 12(12):1239-1249.
36. Sreedhar AS. Hsp90 inhibitors: their effects on redox status and on the cytoarchitecture.
37. Taipale M, Jarosz DF, & Lindquist S. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nature reviews Molecular cell biology 2010; 11(7):515-528.
38. Vogel P (2008) Combinatorial synthesis of linearly condensed polycyclic compounds, including anthracyclinones, through tandem Diels–Alder additions. *Anthracycline Chemistry and Biology I*, (Springer), pp 187-214.
39. Wang Y, Trepel JB, Neckers LM, & Giaccone G. STA-9090, a small-molecule Hsp90 inhibitor for the potential treatment of cancer. Curr Opin Investig Drugs 2010; 11(12):1466-1476.
40. Yang Z-Q, Geng X, Solit D, Pratilas CA, Rosen N, & Danishefsky SJ. New efficient synthesis of resorcinylic macrolides via ynolides: establishment of cycloproparadicicol as synthetically feasible preclinical anticancer agent based on Hsp90 as the target. Journal of the American Chemical Society 2004; 126(25):7881-7889.